# EVALUATION OF NOVEL ENZYME SUBSTRATES FOR THE DETECTION OF COLIFORMS IN WATER SAMPLES

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# Abstract

Possession of specific enzymes can be diagnostic and more reliable than detection of the end products of a metabolic pathway for the enumeration of coliform organisms. A number of chromogenic ß-galactosidase substrates were evaluated with a range of coliform organisms with regard to the intensity of the colour formed upon hydrolysis of the substrate and potential inhibitory effect on coliform growth. A dual substrate membrane filtration medium was developed to detect coliforms and *E. coli* on the same membrane filter, constituting a highly cost-effective method without the need for extensive confirmatory tests. The medium was evaluated with a number of coliform organisms and simulated chlorine-stressed contaminated water samples.

The intense fluorescence of coumarin-based molecules has enabled them to be incorporated into enzyme-based tests for the quantitative assay of indicator bacteria. Several novel derivatives were evaluated in this study and were found to be more promising than the commercially available coumarin giving a combination of greater fluorescence over a broad pH range and reduced growth inhibition with representative coliform strains. On conversion to ß-galactoside derivatives, the substrates were evaluated and incorporated into a miniaturised broth assay format, based on most probable number (MPN).

The chromogenic and fluorogenic substrates described here have been evaluated and have shown their potential as powerful tools in diagnostic microbiology, utilising specific enzymatic activities of coliforms, either in addition to or in place of standard methodologies.

# CHAPTER ONE

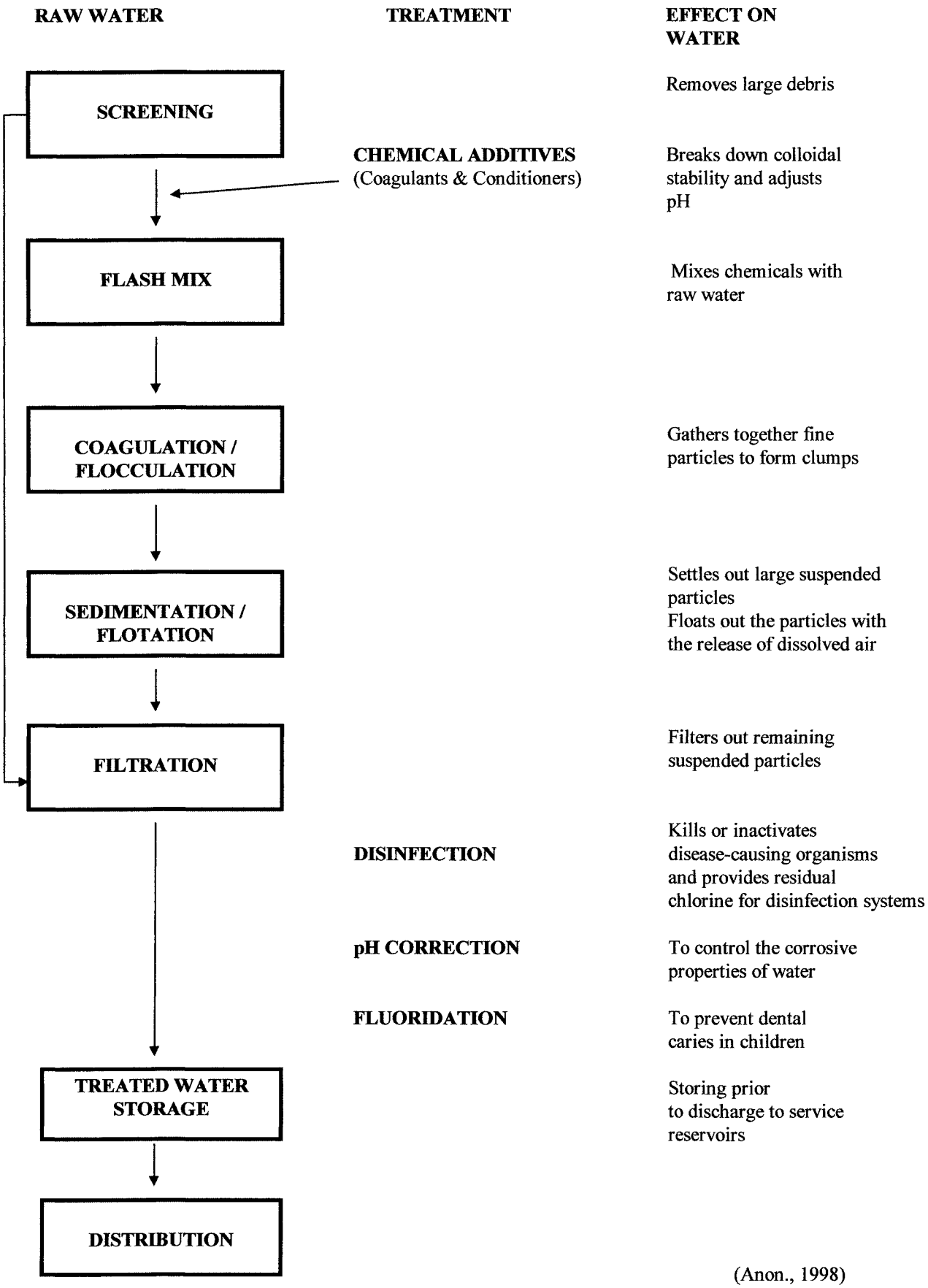
## Introduction

# Background

Water has been recognised as a potential carrier of disease since the beginning of history. The connection between a supply of clean water and the health of an urban population was recognised by the time of the Roman Empire. Ancient civilisations practised water treatment, as evidenced by Egyptian inscriptions and Sanskrit writings. Sand filtration was in use by some cities in the 16$^{th}$ century (Berger *et al.*, 1992). Much of the technology for the protection of the water supply was subsequently lost until the middle of the nineteenth century. In 1854 cholera was linked with the consumption of contaminated waters when it was discovered that a drinking water well in London was in close proximity to a sewer (Dadswell, 1990). This led to the appreciation that protection of water sources, effective water treatment and distribution systems, well-maintained sewers, comprehensive wastewater treatment facilities and pollution control measures were vital factors in improving the health of a community (Tebbut, 1990).

Most water supplies are obtained from surface water sources such as rivers and lakes, or from ground water sources known as aquifiers. At present, municipal water supplies in the UK are usually purified by a process that consists of at least three or four steps (see Figure 1.1). Coagulation is usually the primary processing step, used to hasten the agglomeration of fine particles (Geldreich, 1996). If the raw water contains a great deal of suspended material, it is often first routed to a sedimentation basin and held, so that sand and other very large particles can settle out.

**Figure 1.1: Typical water treatment process**




(Anon., 1998)

The partially clarified water is then mixed with chemical flocculants such as aluminium sulphate (alum) or sodium aluminate and moved to a settling basin, where more materials precipitate out. This process removes some micro-organisms, organic matter, toxic contaminants and suspended fine particles. After these steps the water is further purified by passing it through a filtration unit, for example, a rapid sand filter which depends on a physical trapping of fine particles, is often used for this purpose. This filtration step removes up to 99% of the remaining bacteria (Prescott *et al.*, 1993). After filtration the water is treated with a disinfectant. Various types of disinfection are currently used, including chlorine, chloramines (chlorine combined with ammonia or organic amines), ozone, chloride dioxide and ultraviolet light, although chlorination, introduced in the first decade of the 20$^{th}$ century, continues to be the primary disinfectant used in water supply treatment (Akin *et al.*, 1982).

As indicated above, the final treatment process for drinking water is disinfection, intended to inactivate any pathogenic micro-organisms that have penetrated the filtration process (Tebbut, 1990). The effectiveness of disinfection is a function of the types of micro-organisms to be inactivated, the quality of the water and the nature and concentration of the disinfectant, the exposure or contact time and the temperature of the water (Akin *et al.*, 1982). Chlorine is generally added in sufficient quantities to meet the chlorine demand (i.e.: the tendency of organic substances and ammonia to react with chlorine) and still leave a sufficient residual concentration of chlorine, which is a significant advantage. Other advantages of chlorination include low cost, ease of handling, ease of measurement and ease of availability (Geldreich, 1996). The chlorine dose must be large enough to leave residual (free) chlorine at a concentration of 0.2 to 2.0 mg/l (Prescott *et al.*, 1993). It has been estimated that 3 to 100 times more

chlorine is required to inactivate enteric viruses than is needed to kill coliform bacteria; for this reason a residual free chlorine level of at least 0.3 mg/l is frequently used (Geldreich, 1996).

The tendency of chlorine to combine with organic substances in the water is a major shortcoming of this form of disinfection as some of the by-products are toxic (e.g. trihalomethanes such as chloroform) . Chloramines do not create toxic by-products but their microbial inactivation is not as good as chlorine. Chlorine dioxide also has the advantage of not forming toxic by-products; however, its intermediate products such as chlorite and chlorate are toxic (Berger *et al.*, 1992). Ozonation is an effective method of disinfection, and is widely used in water treatment in Europe, particularly Ireland, France and Switzerland (Anon., 1998). An advantage of ozonation is that fewer toxic by-products have been identified than with chlorine. It is, however, more expensive, cannot be stored, and does not leave a residual effect to inactivate micro-organisms throughout the system (Acher *et al.*, 1994).

Ultraviolet (UV) light is sometimes used in small and medium-scale systems, especially individual domestic systems. UV radiation, produced by very low pressure mercury vapour lamps, disinfects clear water passed over the lamps in a thin layer. This technique is not as widely used as chemical disinfection, although the technology has developed rapidly in recent years (Anon., 1998). The optimal UV wavelength for biocide activity is 254 nm (Acher *et al.*, 1994). No toxic by-products are formed by UV disinfection but it does not leave any disinfectant residual. The effectiveness of this form of disinfection is significantly reduced by a range of factors including high turbidity and high organic content of the water (Fujioka and Narikawa, 1982).

Water temperature can affect disinfection rates - microbial inactivation rates decrease as water temperature decreases and low water temperature represents a worst case condition for chemical disinfection. Another factor to be considered is pH, which in most water systems is kept in the range 7-9. With chlorination, pH values below 7 result in chlorine converting to hypochlorous acid (HOCl), whereas high pH values (8-10) result in the formation of hypochlorite ions ($OCl^-$). HOCl is a significantly more powerful bacteriocide than $OCl^-$ (Anon., 1998) and so is favourable for rapid inactivation.

Unfortunately, purification steps, including chlorination, often do not consistently and reliably remove viruses and protozoal parasites, such as cysts of *Giardia* and *Cryptosporidium*. The protozoan *Cryptosporidium parvum* was recognised as a waterborne pathogen in 1983, and between 1983 and 1986 some 4000 infections were reported in Britain (Galbraith *et al.*, 1987). In the UK *Cryptosporidium* is the fourth most common cause of waterborne-related diarrhoea (Badenoch, 1990). *Giardia lamblia* has been the most frequently identified etiologic agent in United States waterborne disease outbreaks since 1978 (Herwaldt *et al.*, 1992). More consistent removal and inactivation of *Giardia* and *Cryptosporidium* cysts can be achieved with a slow sand filter, which is more a biological rather than a physical treatment, involving the slow passage of water through a bed of sand in which a microbial layer covers the surface of each sand grain; waterborne micro-organisms are removed by adhesion to the surface microbial layer. Coagulation and filtration can reduce virus levels by about 90 - 99%: further inactivation of viruses by chemical oxidants, high pH and photo-oxidation may yield a reduction as great as 99.9% (Berger *et al.*, 1992).

## Microbiological indicators of faecal contamination

Ideally, specific detection of the various pathogenic agents of waterborne disease would be the most direct approach in monitoring and reducing their prevalence in water supplies. A wide range of viral, bacterial and protozoan diseases result from the contamination of water with human faecal wastes. Although many of these pathogens can be detected directly, the variety of potential pathogens makes a search for all, especially on a routine basis, extremely time-consuming, expensive and difficult (Berger *et al.*, 1992). Therefore, searching for a range of pathogenic bacteria directly is not a practical safeguard for a water supply, especially when most screening tests are expected to be negative.

Environmental microbiologists have generally used specific indicator organisms as an indication of possible water contamination by human pathogens, due to the problems of detecting specific enteric pathogens. An 'ideal' indicator organism (bacterium) to use in sanitary microbiology would fulfil all of the following criteria:

- The indicator organism should be suitable for the analysis of all types of water: tap, river, ground, impounded, recreational, estuary, sea and waste waters.
- The indicator organism should be present whenever enteric pathogenic micro-organisms are present.
- The indicator organisms should survive longer than the hardiest enteric pathogen.

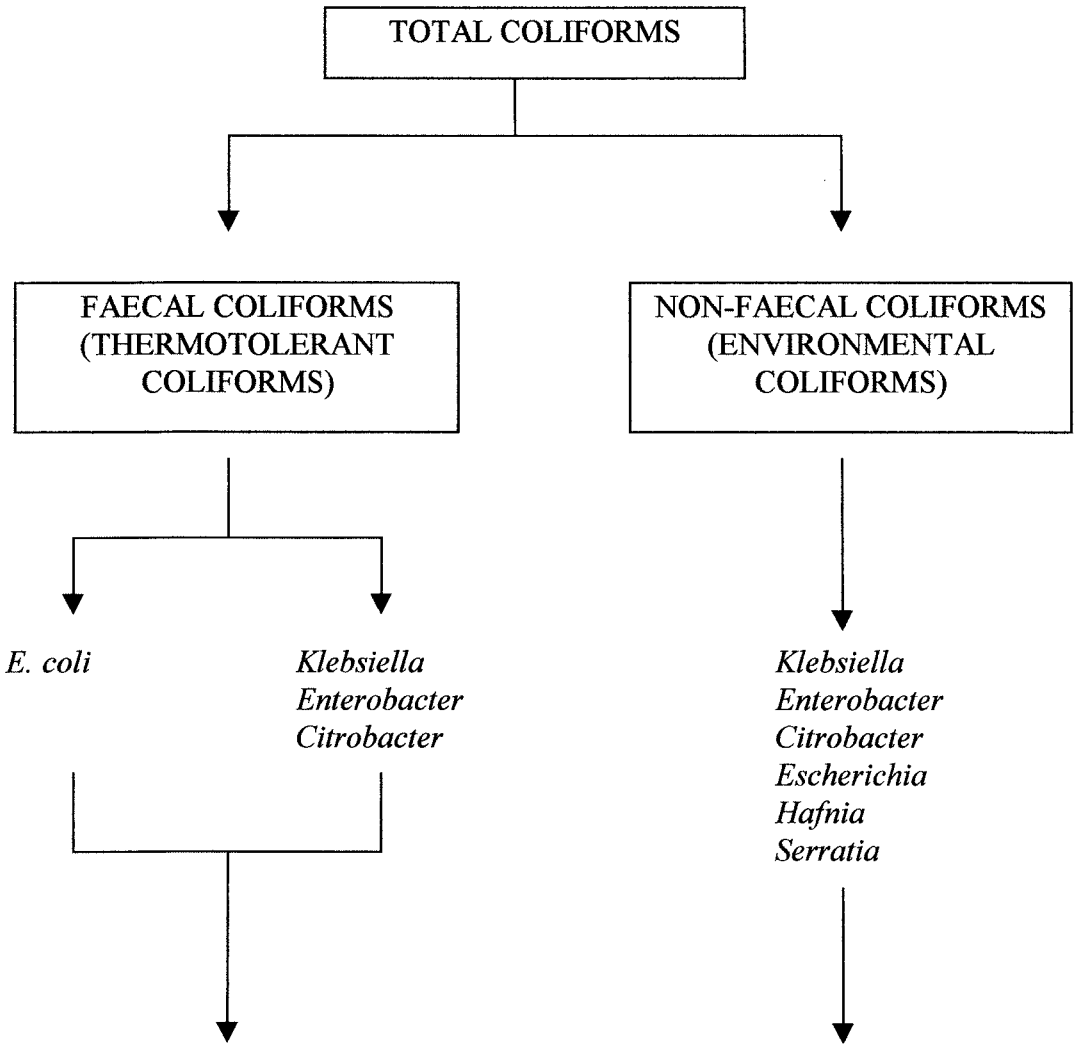
- The indicator organism should not be present in the absence of faecal contamination and should not reproduce in the contaminated water as this would result in an inflated estimate of its presence.
- The assay procedure for the indicator should have great specificity, i.e. there should be confidence that a positive result is indicative of the particular micro-organism to be detected, rather than a range of micro-organisms with similar characteristics. In addition, the procedure should have a high sensitivity, i.e.: it should be able to detect low levels of the indicator.
- The testing method should be easy to perform.
- The indicator organism should be harmless to humans.
- The level (number) of the indicator organism in contaminated water should have some direct relationship to the degree of faecal pollution (Oliveri 1982; Berger *et al.*, 1992)

In practice there is no organism or group of organisms which meets all of the above requirements. The most important sanitary indicators employed world-wide are members of the coliform group, either total coliforms, faecal coliforms, or both: these bacteria fulfil many of the above criteria and have been the principal means of determining water quality for nearly a century (Gleeson and Gray, 1997).

In the earliest studies, bacteriologists found that human faeces contained large numbers of aerobic, Gram-negative organisms which were subsequently found to be tolerant of bile salts, facultatively anaerobic, capable of growth and fermentation of lactose at 37°C (Anon., 1994). Among this group was *Bacterium coli,* isolated from human faeces in 1885 by Escherich and subsequently re-named *Escherichia coli* (Dadswell, 1990). The coliforms, including *Escherichia coli*, are members of the family

Enterobacteriaceae: these bacteria make up approximately 10% of the intestinal micro-organisms of humans and other animals (Finegold and George, 1989), are generally non-pathogenic and have found widespread use as indicator organisms (see Figure 1.2). Coliforms are defined differently, depending on the method used for their detection. However, the broadest definition is as follows: Gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties, able to ferment lactose at 35-37°C with the production of acid, gas and aldehyde within 24-48 h. They are also oxidase-negative, non-spore-forming and display β-galactosidase activity (Anon., 1993a). Coliforms include most species of the genera *Enterobacter*, *Klebsiella*, *Citrobacter*, *Escherichia*, *Hafnia*, *Yersinia* and *Serratia* (Anon., 1994). The 'total coliform' count is used to determine the overall effectiveness of water treatment, to monitor the integrity of the underground pipe network, and also as a screen for fresh faecal contamination. Treatment and other wastewater system practices that provide coliform-free water should also reduce pathogens to minimal levels. A major shortcoming of the use of 'total coliforms' as an indicator is that they are poor for predicting the potential presence of some pathogenic protozoa and viruses, since coliforms are less resistant to disinfection than these micro-organisms (Berger *et al.*, 1992).

**Figure 1.2: Coliform groups relevant to water microbiology**



Faecal coliforms are a subset of the coliform group, primarily including *E. coli* and a few thermotolerant strains of *Klebsiella, Enterobacter* and *Citrobacter*. They are defined as being able to ferment lactose at 44°C, i.e. they are more thermotolerant than other coliforms. The excreta of man and animals, including fish and birds, contain enormous numbers of the bacterium *E. coli* as a natural inhabitant of the intestine, approaching billions per gram (Twort *et al.*, 2000). Faecal coliforms and *E. coli* are more suitable and specific indicators of fresh faecal contamination than other coliforms, and most waterborne pathogenic bacteria are associated with fresh faeces. However, the 'total coliform' count is a more suitable indicator to determine the vulnerability of a treated water system to faecal contamination, especially in the absence of faecally contaminated samples at the time and location of sample collection. This is because coliforms are usually present at much higher concentrations in source waters and they are relatively more resistant to chlorine and other environmental stresses (Berger *et al.*, 1992).

In addition to those indicators described above, other drinking water indicator micro-organisms have been used or suggested, usually as a supplement to the principal indicators. These include faecal streptococci (enterococci), coliphages, *Clostridium perfringens* and *Bacteroides*. The use of enterococci as an indicator of faecal contamination of recreational water was recommended by the US Environmental Protection Agency in 1986; this was based on studies which demonstrated that enterococci had a strong direct relationship to swimming-associated illness (Messer and Dufour, 1998). Several chemical indicators have also been proposed, for example faecal sterols, assimilable organic carbon and disinfectant residuals (Berger *et al.*, 1992). Faecal sterols such as coprostanol and cholesterol have been proposed to

indicate human faecal contamination of water systems (Dutka *et al.*, 1974). Faecal sterols do not appear to be affected by chemical disinfectants, which is an advantage. However, they require an elaborate extraction process which would not be feasible on a routine basis (Waite, 1984; Gleeson and Gray, 1997).

The use of method-related definitions of microbiological indicators is becoming largely obsolete, with the development of methods that do not rely on characteristics such as the production of acid and gas from lactose (Gleeson and Gray, 1997). A particular problem with this characteristic is that the process begins with the cleavage of lactose by the enzyme β-galactosidase into glucose and galactose. The expression of the gene encoding for the production of β-galactosidase can be affected by many factors so that the organism may or may not ferment lactose sufficiently to register as a lactose fermenter under test conditions (Anon., 2000). It is now widely accepted that new definitions of the coliform group should be based on the possession of the β-galactosidase gene. Similarly for *E.coli*, as well as being able to ferment lactose, producing acid and gas at 44°C, it is also able to produce indole from tryptophan. However, most strains (90-97%) also possess β-glucuronidase, providing an alternative definition based on the possession of this enzyme (Anon., 1983; Anon., 2000).

## Standards of bacteriological quality

There are four main standards of drinking water quality (see Table 1.1):

I) EU legislation;

II) UK regulations, which are derived from (I);

III) US (EPA) standards;

IV) WHO recommendations.

### I) EU Legislation

The EU Drinking Water Directive was implemented in July 1980. This Directive applies to all water supplied for human consumption within the European Community and also to water used in processing food products intended for human consumption (EC Directive L229/11, 1980; updated EU 1995). The criteria on which this Directive is based were largely drawn from WHO guidelines. The Directive lists 66 parameters which are classified into six groups: (a) organoleptic, (b) physicochemical, (c) substances undesirable in excessive amounts, (d) toxic substances, (e) microbiological and (f) minimum concentrations for softened water (Anon., 1980). A number of microbiological parameters are set by the Directive using indicator organisms. It is recommended that bacteriological analysis is done by membrane filtration (see later) for total coliforms, faecal coliforms and faecal streptococci (enterococci), and by multiple tube method (see later) for sulphite reducing clostridia. The EU Directive does not require either viruses or protozoan pathogens (cysts or oocysts) to be routinely measured and, although it makes it clear that water intended for human

consumption should not contain any pathogenic organisms, in terms of regulated parameters it relies totally on the indicator organisms mentioned above.

## II) UK regulations

In England and Wales the implementation of the EU Drinking Water Directive began in 1989 with the enactment of the Water Supply (Water Quality) regulations (Gleeson and Gray, 1997). There have been numerous problems associated with the implementation of the Drinking Water Directive (Crowley and Packham, 1993), including scientific practicality, assessment of the priority of standards set and, as comprehensive statistics on drinking water are not available throughout the EU, comparisons are difficult to make. In 1995 a revised, simplified Drinking Water Directive was published proposing three microbial, 26 chemical and 16 indicator parameters (Anon., 1995). The revised Directive gives separate maximum permissible concentrations for the microbial parameters for both tap and bottled waters (excluding natural mineral waters which have their own EU Directive). Maximum permissible numbers are given for *E. coli*, faecal streptococci and sulphite reducing clostridia. *Pseudomonas aeruginosa* was also included for the first time although, as before, no guideline values were provided for specific viruses, protozoa or bacterial pathogens.

## III) US Legislation

The US Environmental Protection Agency (EPA) publishes enforceable regulations under the Safe Drinking Water Act, which was passed by the United States Congress in 1974 and subsequently amended several times. Regulations under this Act apply to

all public water systems that regularly serve 25 or more people on at least 60 days of the year, or that have 15 or more service connections (Masters, 1991). The number of public water systems in the US is approximately 200000 (Berger *et al.*, 1992). An amended Act in 1986 required the US EPA to specifically regulate for *Giardia*, viruses, *Legionella*, heterotrophic bacteria and turbidity, in addition to coliform counts (Anon, 1993a). The Total Coliform Rule was revised in June 1989 and came into practice at the end of 1990. This regulation sets a maximum contaminant level (MCL) for total coliforms and specifies that one of the following techniques must be used when analysing a water sample: 10-tube multiple tube fermentation technique, the membrane filtration technique, the Presence-Absence coliform test or the minimal medium ONPG-MUG (*ortho*-nitrophenyl-ß-D-galactoside/4-methylumbelliferyl-ß-D-glucuronide) test (Edberg *et al.*, 1989; Berger, 1992).

**IV) WHO recommendations**

These guidelines for drinking water quality are used universally and are the basis for both EU (UK) and US legislation. A revised set of guidelines from the original set published in 1984 came into force in 1992, which included microbiological, chemical and radiological parameters. There are no specific guideline values for either viruses or parasites; instead guideline criteria are based on the likely viral content of source waters and the degree of treatment necessary to ensure that volumes of water have a negligible risk of containing viruses (Anon., 1993b).

**Table 1.1: Bacterial Standards (Anon., 1995; Anon., 1993b; Twort, 2000)**

| | WHO GUIDELINES | EU DIRECTIVE | UK REGULATIONS | US EPA |
|---|---|---|---|---|
| Faecal coliforms | 0/100 ml | 0/100 ml | 0/100 ml | 0/100 ml |
| Total coliforms | 0/100 ml in 95% of samples in the case of large supplies, throughout preceeding 12 months | 0/100 ml in 95% of samples provided sufficient numbers of samples taken | 0/100 ml in 95% of samples in preceding year, or if less than 50 samples taken, the last 50 samples | 0/100 ml in 95% of samples or if less than 40 samples taken per month not more than 1 positive per month |
| Faecal streptococci | - | 0/100 ml | 0/100 ml | - |
| Sulphite-reducing Clostridia | - | MPN less than 1/20 ml sample | MPN less than or equal to 1/20 ml | - |
| Total colony or bacteria count at 22°C or 37°C | - | guide level <10/ml at 37°C and <100/ml at 22°C | 'no significant increase over that normally observed' | - |

## Environmental damage to micro-organisms and implications for the resuscitation and detection of indicator bacteria

Enteric bacteria that are either pathogenic or used as sanitary indicator organisms are not well adapted to conditions within aquatic systems. Research has shown (McFeters *et al.*, 1986; McFeters, 1989) that the majority of coliforms found in drinking water are injured (McFeters and Singh, 1991). Injury has been described as sublethal physiological damage resulting from the exposure of micro-organisms in drinking water to various physical, chemical and biological factors which affect their ability to grow on selective media that are satisfactory for the growth of normal (undamaged) cells (LeChevallier *et al.*, 1985; McFeters, 1990; Brenner *et al.*, 1996). Sublethal injury was first associated with the suppression of waterborne indicator bacteria when it was noted that coliform enumeration data from waters containing toxic wastes or chlorine were consistently higher by the multiple tube fermentation (MPN) method than by the membrane filtration (MF) procedure (Hurst, 1977; Camper and McFeters, 1979; Tillet *et al.*, 1988). Studies have since demonstrated that in comparison to undamaged cells, a variety of genera of injured coliforms can give reduced plate counts on a range of selective media used for the enumeration of Gram-negative enteric bacteria (McFeters *et al.*, 1982; LeChevallier *et al.*, 1983, 1984; McFeters and Singh, 1991). Failure to detect such injured coliforms could lead to an over-optimistic estimate of the safety of water where the pathogens may still exist (Calabrese and Bissonette, 1990a).

Factors present in drinking water that can cause injury include: antimicrobial chemicals and other disinfectants, particularly chlorine and other biocides; low concentrations of

metals, particularly copper and zinc; extremes of temperature and pH; and also, interactions with other bacteria (Geldreich *et al.*, 1978; Zaske *et al.*, 1980b; McFeters *et al.*, 1982). Both UV and visible components of sunlight have a biocidal effect on bacteria. However the damage is more pronounced in seawater than in freshwater due to more favourable conditions in freshwater, such as lower salinity and the presence of light-absorbing substances including humic and fulvic acids (Davies and Evison, 1991). After exposure to these damaging factors, coliforms are uniquely susceptible to ingredients such as desoxycholate, bile salts and surfactants that are found in most selective media for coliforms, and this has led to cited outbreaks of waterborne disease linked to water sources where coliforms were not found (e.g. Boring *et al.*, 1965, Seligman and Reitler, 1965).

**Damage by disinfectants**

The widespread use of aquatic disinfectants in combination with the numerous other stresses potentially found in water indicate that all disinfected systems, as well as many that are non-treated, are likely to include factors that can injure enteric bacteria, including faecal indicators. Of the potential stresses, sublethal levels of aquatic disinfectants are usually the primary agents in treated aquatic systems such as potable (drinking) water (McFeters and Singh, 1991). The earliest research on the physiology of bacterial chlorine injury was reported by Green and Stumpf (1946) who showed that chlorine acted as an inhibitor of glucose oxidation in cells. Chlorine is now known to specifically affect the cytoplasmic membranes of cells, and thus membrane-related functions (e.g. oxidative phosphorylation), and also disrupt protein synthesis, affecting nucleic acid metabolism, creating chromosomal aberrations and compromising nutrient

transport (Brenner *et al.*, 1996; Anon., 1998). Reduction in oxygen uptake, cellular ATP concentration and oxidative phosphorylation have been shown to occur in *E. coli* as the result of chlorine-damaged membranes (Calabrese and Bissonette, 1990a). Research carried out by LeChevallier *et al.* (1985) found that a free chlorine residual of 0.25 - 0.5 mg/l caused an injury rate greater than 90% in coliforms, whereas levels of 0.9 - 1.5 mg/l were required to give comparable damage with some other Enterobacteriaceae such as *Yersinia enterocolitica*, *Salmonella typhimurium* and *Shigella* spp. This suggests that coliform bacteria and various potential waterborne pathogenic micro-organisms may differ in their sensitivities to chlorine injury.

**Damage by sunlight**

The detrimental effects of sunlight are believed to play an important role in the microbial ecology of the upper regions of aquatic environments (Whitelam and Codd, 1986). Moreover, sunlight rapidly reduces the number of faecal indicators and enteric pathogenic bacteria in both freshwater, brackish and seawater (Chamberlain and Mitchell, 1978; Davies and Evison, 1991; Joyce *et al.*, 1996; Burkhardt *et al.*, 2000). Indeed, waste stabilisation ponds have been used for some time in sewage treatment systems and are very effective at removing enteric pathogens (Curtis *et al.*, 1992). The performance of a waste stabilisation pond is known to vary seasonally: using partial regression techniques, very good inverse linear relationships between the number of faecal coliforms in a pond and the direct effects and indirect (e.g. via increases in algae and pH) effects of sunlight have been found (Troussellier and Legendre, 1989).

In order for light to damage a micro-organism the light must first be absorbed by a chemical sensitiser; these can come from within the cell (endogenous) or from outside the cell (exogenous). An efficient sensitiser may enter into an excited state for long enough to react with other molecules and initiate damaging photosensitisation reactions (Whitelam and Codd, 1986). These reactions are usually much more injurious in the presence of oxygen (Curtis *et al.*, 1992). When the excited sensitiser reacts with oxygen, a number of reactive species may be formed including: singlet oxygen; superoxide; hydrogen peroxide; and hydroxyl radicals (Whitelam and Codd, 1986). As with chlorine, the cytoplasmic membrane is the most likely target for light-mediated damage at the wavelengths found in the visible spectrum of sunlight (400 - 750 nm), also known as photosynthetically-active radiation (Muela *et al.*, 2000). Additional damage to DNA is usually by UV-A and UV-B wavelengths between 280nm and 400 nm (Davies and Evison, 1991). Wavelengths less that 280 nm (UV-C) are effectively absorbed by the ozone layer and so do not represent a significant proportion of the solar spectrum that reaches the Earth (Whitelam and Codd, 1986). Recent research has shown an increase of up to 10-20% of UV-B radiation for both north and south temperate latitudes since the initial report of the depletion of the ozone layer, and this may increase the significance of this component of sunlight (Muela *et al.*, 2000).

There is a linear relationship between oxygen concentration and sunlight-induced damage to bacteria (Webb and Lorenz, 1970). Some studies have shown that faecal bacteria are not always fully inactivated in sunlight (Miller, 1988, MacKenzie *et al.*, 1992), and have observed different rates of inactivation in aerobic and anaerobic waters (Reed, 1997a, 1997b). Further studies have clearly indicated that solar

inactivation of faecal indicator bacteria is fully effective only under aerobic conditions (Reed *et al.*, 2000).

**Resuscitation techniques**

As well as the damaging environmental factors already mentioned, once a water sample reaches the laboratory, manipulations involving exposure to diluents, membrane filtration and selective media may cause further underestimation of bacterial numbers. In an effort to improve detection of sublethally-injured micro-organisms several procedures have been suggested to 'resuscitate' stressed indicator bacteria (e.g. coliforms) before they are isolated using selective and differential media. The addition of catalase to various culture media has been recommended by many (e.g. Baird-Parker and Davenport, 1965; Martin *et al.*, 1976; Rayman *et al.*, 1978; Calabrese and Bissonette, 1990a, 1990b). This is based on the principle that standard recovery procedures for coliforms utilise aerobic incubation on selective media, requiring the cell to function under respiratory conditions involving the reduction of oxygen to water. Catalase is an enzyme produced by most aerobic micro-organisms for the degradation of toxic hydrogen peroxide, which is a by-product of oxidative metabolism: the enzyme therefore prevents the accumulation of hydrogen peroxide to which injured bacteria become increasingly sensitive (Mossel *et al.*, 1980). More recent recovery techniques involving catalase coupled with sodium pyruvate as a scavenger of reactive oxygen free radicals have been recommended resulting in significantly greater detection of faecal coliforms (Calabrese and Bissonnette, 1990a, 1990b).

When using the MPN (most probable number) technique (see later) the addition of time-release capsules containing selective ingredients to the assay has been suggested (Lantz and Hartman, 1976). This would allow for repair of injured cells before the selective agent in the medium reaches inhibitory levels. Coliform suppression is particularly common during the confirmatory (second) stage of the MPN technique due to the inhibitory nature of the confirmatory broth, particularly with chlorinated effluents (Evans *et al.*, 1981). In the early stages of membrane filtration (MF) development it was recognised that MF had a lower recovery rate that MPN (Thomas *et al.*, 1956) and whilst some of these differences can be attributed to the mathematical bias of the MPN technique, physiological injury of bacteria may well provide some explanation for the poorer growth on the surface of filter membranes (Camper and McFeters, 1979; LeChevallier *et al.*, 1984).

Injured bacteria have been shown to have reduced intracellular ATP, lower glucose transport and utilisation, reduced aerobic respiration, decreased production of secondary metabolites, lower resistance to disinfection, and a reduction in cell size (Singh and McFeters, 1992). Moreover, even short-term exposure to environmental stress can cause cellular envelope damage in bacteria, increasing their sensitivity to selective agents in agar-based isolation media (Zaske *et al.*, 1980b). To accommodate this, an enrichment step can be included in the MF technique to allow injured cells to resuscitate. This consists of placing membranes on a less selective medium immediately after filtration, for example, on lauryl tryptose lactose broth, prior to transfer to the final selective medium. However, the addition of this resuscitation step has certain limitations in that more time, equipment and labour are required for analysis. It has also been observed (Evans *et al.*, 1981) that the inclusion of this step into the MF

technique served no advantage in terms of increased coliform recovery with untreated surface water or chlorinated drinking waters. In Standard Methods for the Examination of Water and Wastewater 15th edition (Anon., 1976b), a section describing various resuscitation techniques was added under the heading 'Stressed Organism Recovery'.

Injury is an important factor in underestimating numbers of waterborne indicator bacteria, which may lead to inaccurate public health assessments. Extensive work has been carried out with enteric pathogens such as enterotoxigenic *E. coli*, *Y. enterocolitica*, *S. typhimurium* and *Shigella* spp., to investigate the persistence of specific virulence-related activities after injury (LeChevallier *et al.*, 1985). The nature and extent of loss varies with the stress and the organism. However, recovery from injury, either *in vivo* or *in vitro*, is accompanied by renewed expression of the virulence-associated activities lost with injury (McFeters and Singh, 1991). This indicates that injured pathogens ingested from the aquatic environment retain the phenotypic potential required to cause enteric illness, and thus an understanding of their presence is important in the task of ensuring water potability.

**Viable but non-culturable micro-organisms**

Nutrient-limited growth is the normal state for most micro-organisms in the environment. In aquatic systems, nutrients are usually absorbed until concentrations decrease to levels that are sufficient only for the growth of those organisms with good transport systems, or of ones that can grow very slowly. Seasonal variations in temperature have also been observed as a factor in the ability of an organism to be

cultured on routine bacteriological media (Oliver, 1995). The microbial cell can be thought of as being in a dynamic state, adapting to shifts in environmental conditions. These adaptive capabilities account for the apparent ease with which micro-organisms respond to culture conditions in the laboratory, which are often radically different from the natural habitat of the organism (Roszak and Colwell, 1987).

The question of viability has been extensively discussed (Postgate, 1969; Mossel and Van Netton, 1984; Flint, 1987; McKay, 1992; Barer *et al.*, 1993; Bogosian *et al.*, 1996, 1998; Barer and Harwood, 1999). Heinmets *et al.* (1953) observed that organisms considered to be non-viable because of their failure to grow in a complete medium could, nonetheless, grow in the same medium after a short incubation period in the presence of suitable metabolites, e.g. 0.1% pyruvate, acetate or oxaloacetic acid. This supported the theory that synthetic and metabolic processes continue in the absence of cell division under stressful conditions (Roszak and Colwell, 1987; Davies *et al.*, 1995). Bretz (1962) reported from studies of slide cultures that some cells, which he called moribund, were observed only to swell during the same period required for other cells to divide. These moribund cells eventually formed microcolonies and were therefore considered viable.

There are numerous methods to differentiate living bacteria from dead bacteria by microscopic examination of cells. The redox dye 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) has been used for direct epifluorescent microscopic enumeration of respiring bacteria in environmental samples (Rodriguez et al., 1992). The acridine orange direct-count, has been widely used and is accepted as being reliable for the direct enumeration of total bacterial populations (Daley and Hobbie, 1975; Hobbie *et*

*al.*, 1977; Roszak and Colwell, 1987; Ravel *et al.*, 1995). The basis of the test is that the acridine orange dye fluoresces red in association with RNA and green when in association with DNA. A high RNA/DNA ratio indicates active metabolism, therefore, red fluorescence correlates with active cells and green fluorescence correlates with inactive cells. However, a major problem with this method is that factors other than nucleic acid ratio affect the fluorescence produced by acridine orange, such as pH and incubation time (Roszak and Colwell, 1987).

A method was developed by Kogure *et al.* (1979) in an attempt to overcome the problem of underestimation of viable cells by plate counts, and overestimation by direct microscopy. To estimate directly the viable bacteria present in seawater, samples were pre-incubated with small quantities of nalidixic acid and yeast extract. Nalidixic acid is a specific inhibitor of DNA synthesis, preventing cell division in Gram-negative bacteria by inhibiting DNA replication and thus preventing cross-wall formation (Goss *et al.*, 1965). However, other synthetic pathways continue to function, resulting in elongation of the metabolically-active cells. This direct viable count enumerates all cells which are actively-growing as well as dormant cells which are physiologically responsive. However, some Gram-negative bacteria are resistant to this particular antibiotic, potentially causing underestimation of viable populations (Roszak and Colwell, 1987).

Indicator bacteria could potentially be underestimated if bacteria enter into the viable but non culturable (VNC) state. As previously mentioned, without the inclusion of a 'recovery period', either in the presence of metabolites or at a reduced incubation

temperature, indicator organisms may fail to grow, leading to the assumption that they are not present.

## Traditional methodologies for testing water supplies

As discussed previously, the detection of coliforms has traditionally been based on the production of acid and gas in lactose-based media. There are essentially two standard methods for the enumeration of coliforms from drinking water, both of which rely on the above characteristics, and these are i) most probable number (MPN) method and ii) membrane filtration (MF).

### i) The Multiple Tube method (Most Probable Number method)

This method was first described by McCrady (1915) and is based on the principle of dilution to extinction (Mara, 1974). Measured volumes of one or more dilutions of a sample are added to a series of tubes containing a suitable liquid differential medium. If a viable micro-organism is present it will grow, producing a characteristic change in the medium. A two-stage technique is used, the first, presumptive stage is an estimation of the total coliforms in a sample, this is followed by the second, confirmatory stage. In the presumptive stage a lactose-based medium is used with a pH indicator to detect acid production and an inverted Durham tube to detect gas production. In the UK, the use of minerals modified glutamate medium (MMGM) is recommended (Anon., 1994), whereas in the USA lauryl tryptose lactose broth (LTLB) is widely used (Anon., 2000). Both media have superseded the use of MacConkey broth (Gleeson and Gray, 1997) which was made from peptone and bile salts, both of which were found to vary significantly from batch to batch in their nutrient and inhibitory properties (Mara, 1974). Volumes and dilutions of sample are

selected, depending on the suspected quality of the water, so as to maximise the chance of having some positive and some negative reactions in the dilution series (Anon., 1994). Once inoculated, tubes are incubated at 37°C (Anon., 1994) or at 35°C (Anon., 2000) and examined after 24 and 48 hours, recording all positive tubes. The resulting pattern of positive (gas production) and negative results in the dilution series is then used to obtain a statistical estimate - the most probable number (MPN) - which is calculated by reference to probability tables devised by McCrady (1915). MPN is expressed as the number of cells per 100 ml sample.

The confirmatory stage amounts to the sub-culturing of any tubes which show a positive reaction, either acid or gas production at the presumptive stage, to tubes of confirmatory media, either brilliant green lactose bile broth (BGLBB) with incubation at 37°C for 48 hours (Anon., 2000) or lauryl tryptose lactose broth (LTLB) with incubation at 35°C for the same period (Anon., 1994). This confirms the presence of coliforms in the original sample, determining the MPN. A number of techniques can be used to differentiate faecal coliforms from other coliforms when using elevated temperatures (44°C) and the MPN method, including: the Eijkman test; lauryl tryptose mannitol broth; the IMViC tests - a set of tests for indole formation, the Methyl Red and Voges Proskauer reactions, citrate utilisation; and the production of gas from lactose at 44°C. Use of elevated temperatures confirms the presence of thermotolerant (faecal) coliforms, such as *E. coli* strains (Anon., 1994; Anon., 2000).

It is generally accepted that the MPN procedure is a method of low precision, which is to be expected of a procedure which does not use direct counts. Moreover several reports have observed the failure of the method to detect coliforms in drinking and

other waters (Geldreich *et al.*, 1972, Seidler *et al.*, 1981). A number of reasons have been suggested, including the unsuitability of the medium used for the recovery of injured coliforms, and this will be discussed later.

The presence-absence (P-A) test, which is a modification of the MPN procedure, can be used for coliform detection (Clark, 1968). For this test, a larger water sample (50-100 ml) is incubated in a single culture bottle with lauryl tryptose broth containing bromocresol purple indicator. Samples are incubated at 35°C for up to 48 hours. The P-A test is based on the assumption that no coliforms should be present in 100 ml of drinking water. A positive test creates acid from the fermentation of lactose, shown by the indicator becoming a yellow colour, constituting a positive presumptive test. Confirmation of the presumptive result consists of removing a small inoculum from a positive sample to a tube of brilliant green bile lactose broth with confirmation based on acid and gas production, within 48 hours at 35°C.

Various comparative studies (Jacobs *et al.*, 1986; Caldwell and Monta, 1988; Rice *et al.*, 1989) have observed more sensitive coliform detection with the P-A test, when compared to MPN and MF techniques. The P-A test has been used successfully in Canada since 1969 (Clark, 1980) and in the UK a version of the test has been used for many years for the decentralised testing of distribution waters (Furness and Holmes, 1987). P-A is now listed in the USA as an accepted standard method (Anon., 1995). In the UK an extensive evaluation of a range of P-A based methods concluded that a particular P-A test should be validated in each geographical area before use (Gleeson and Gray, 1997), and that there is no P-A test that is best at all locations for both coliforms and *E.coli* (Lee *et al.*, 1995).

**ii) The Membrane Filtration method**

Until the 1950s practical water bacteriology relied almost exclusively on the enumeration of coliforms and *E. coli* by estimating the MPN. However, since then, the membrane filtration (MF) technique has become a common, and often the preferred, method for the evaluation of the microbial content of contaminated water (Burman, 1967). A measured volume of water sample is passed through a membrane filter, which is then transferred to the surface of a solid medium or to an absorptive pad containing the desired liquid medium. Use of the proper medium allows the rapid detection of total coliforms, faecal coliforms, or faecal streptococci by their characteristic colonies. These colonies can then be counted and the number of organisms per 100 ml calculated.

In the UK, Report 71 (Anon., 1994) recommends the use of membrane lauryl sulphate broth (MLSB) for both total and faecal coliforms. This rich medium contains, among other things, lactose (3% w/v) and a pH indicator in the form of phenol red. Gram-positive organisms are inhibited due to the presence of sodium lauryl sulphate (0.1% w/v). Duplicate samples are membrane filtered, one to be incubated at 30°C for 4 hours followed by 37°C for 14 hours for total coliforms, the second to be incubated with the same split incubation times at a temperature of 30°C followed by 44°C (Anon., 1994).

US Standard Methods (Anon., 2000) recommend the use of m-ENDO agar for total coliforms, which again is an extremely rich medium containing lactose and basic fuchsin as the pH indicator. The medium also includes sodium lauryl sulphate and

sodium desoxycholate to inhibit Gram-positive bacteria. Plates are incubated at 35°C for 22-24 hours. For detection of faecal coliforms and *E. coli*, US Standard Methods recommend filtration onto m-FC medium, which incorporates the use of elevated temperatures and the selective action of rosolic acid salt reagent. The specificity of this test is directly related to incubation temperature, therefore to achieve greater control, incubation in a waterbath at 44.5°C for 24 hours is recommended (Anon., 2000).

Lactose fermentation is detected by a reduction in pH, with MLSB producing yellow colonies on a red background, m-ENDO agar producing purple colonies with a metallic green sheen on a pink background, and m-FC producing colonies of various shades of blue. As colours are likely to change on cooling and when left standing, plates should be examined within a few minutes of their removal from the incubator (Gleeson and Gray, 1997). Unlike the MPN test, presumptive results are usually sufficient for the examination of most waters. However, with potable water, confirmatory tests are necessary: any positive colonies on MLSB, or a representative sub-sample of them, are subcultured to tubes of lactose peptone water (LPW) containing an inverted Durham tube with incubation at 37°C for 48 hours (Anon., 1994). For confirmation of faecal coliforms and *E. coli* positive colonies should also be subcultured to tubes of tryptone water containing a Durham tube and incubated at 44°C for 48 hours. Few non-faecal coliforms will be observed on m-FC agar because of its selective action (Anon., 2000). Alternative confirmatory tests include using a short set of rapid tests incorporating assays for cytochrome oxidase (CO), and β-galactosidase activity (ONPG) respectively. Coliform reactions are CO-negative and ONPG-positive within 4 hours incubation of tube culture or spot test procedures (Anon., 1994; Anon., 2000).

The MF method has numerous advantages over the use of MPN technique including: the provision of results in a shorter time (18-24 hours as opposed to 48 hours); processing of larger volumes of sample is possible; simple methodologies are used; it is possible to carry out filtration in the field (Windle-Taylor and Burman, 1964; Grabow and DuPreez, 1979). However, there are also limitations associated with the technique, in particular: MF is unsuitable for turbid waters; operator variations occur in identifying typical coliform colonies; coliform growth on filters may be inhibited by high background bacterial numbers (Gleeson and Gray, 1997). Moreover the sensitivity of the technique can be affected by a number of variables including diluent composition, exposure time, and membrane type (Tobin et al., 1980; McFeters *et al.*, 1982). Despite the widespread use of MPN and MF techniques, it is recognised that both methods have inherent faults: the time taken for an answer can be as long as 72 hours; further tests are required to differentiate coliforms from faecal coliforms; the subjective nature of interpreting results can lead to inaccuracies; stressed and injured bacteria are very difficult to detect without the addition of an enrichment step which significantly increases the time for analysis.

## New techniques for faecal indicator bacteria

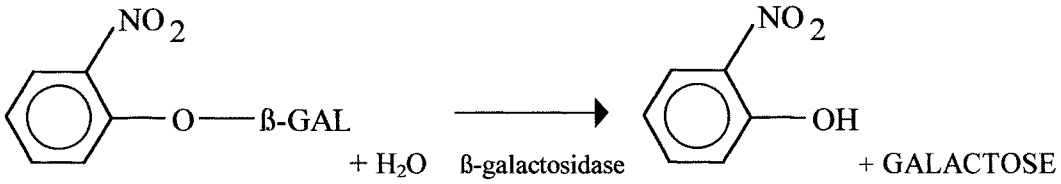
International guidelines controlling drinking water quality adopted by the EC in 1980 were implemented by all member states between 1985 and 1989 (Premazzi *et al.*, 1989). Despite slight variation in the microbiological parameters used by different countries, similar MPN and MF techniques along with similar selective plating procedures have been applied routinely world-wide in both coliform and *E. coli* detection systems. Due to the recognition of the inherent limitations when using these traditional techniques, and especially of the time taken to obtain a response, there has been a growing emphasis on developing rapid methods for drinking water and environmental water samples in order to provide results within a single working day (Frampton and Restaino, 1993). Tests exist which can be performed in less that one hour but they lack specificity, and they may include non-viable cells (Geldreich, 1997; Van Poucke and Nelis, 1997; Davies and Apte, 2000). The alteration of the UK definition of *E. coli*, which no longer refers to the production of gas during the fermentation of lactose (Anon., 1994) has also encouraged these developments. Any potential alternative must involve specificity, sensitivity and precision, with achievement of a test result ideally within a few hours (Geldreich, 1992).

### Utilisation of enzyme-specific substrates

Possession of specific enzymes can be diagnostic and more reliable than detection of the end products of a metabolic pathway, of which the diagnostic enzyme is only one component (Sartory and Watkins, 1999). For example, total coliform detection can be based on the detection of β-galactosidase. This enzyme catalyzes the breakdown of

lactose to galactose and glucose. Chromogens and fluorogens, i.e.: substrates that generate colour and fluorescence respectively upon cleavage by a specific enzyme, have been used extensively for many years to detect and identify bacteria (Bascomb, 1987; Manafi *et al.*, 1991). Coliforms, including *E. coli,* have the ability to degrade the chromogenic substrate *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG) due to their possession of the enzyme β-galactosidase, yielding a yellow-coloured product, *o*-nitrophenol (see Figure 1.3). There are numerous substrates available for β-galactosidase including the chromogen, *para*-nitrophenyl-β-D-galactopyranoside (PNPG) and the fluorogen, 4-methylumbelliferyl-β-D-galactopyranoside (4-MU-GAL).

**Figure 1.3: Hydrolysis of colourless *ortho*-nitrophenyl-ß-D-galactoside (ONPG) to the yellow-coloured *ortho*-nitrophenol.**




A large number of *E. coli* strains, over 94% (Hansen and Yourassowsky, 1984), also possess the enzyme β-glucuronidase and are therefore able to cleave 4-methylumbelliferyl-β-D-glucuronide (MUG), resulting in the formation of the fluorescent product 4-methylumbelliferone which is visible when irradiated with long-wave UV light (Clark *et al.*, 1991). Although the enzyme β-glucuronidase was first discovered in *E. coli* (Buehler *et al.*, 1951), its relative specificity for identifying this

organism was not apparent until Kilian and Bülow (1976, 1979) surveyed the Enterobacteriaceae and the Vibrionaceae and reported that the β-glucuronidase activity was mostly limited to *E. coli* (94-97% of strains positive) and to a lesser degree, *Shigella* spp. (40-67% strains positive), *Salmonella* spp. (17-29%), and a few *Yersinia* spp. also show ß-glucuronidase activity (Frampton and Restaino, 1993). Furthermore, all *Escherichia* spp. other than *E. coli* are reported to be phenotypically β-glucuronidase-negative (Kampfer *et al.*, 1991; Rice *et al.*, 1990, 1991), although this enzyme was previously reported in *E. vernalis* (Holt *et al.*, 1989).

Enzymatic characteristics were first utilised for identification purposes, but they have recently been exploited by newer, rapid methods for environmental testing (Eckner, 1998) with a number of commercial ONPG-MUG preparations available. Tests such as Colilert (Idexx), Coliquick (Hach) and Colisure (Millipore) can be used in MPN or P-A format and involve the addition of a measured amount of sample to tubes containing the powdered ingredients. After incubation at 35°C for 24 hours, test tubes containing coliforms will be yellow, those which also fluoresce under long-wave UV light are presumed to contain *E. coli* (Edberg *et al.*, 1988b, 1990). If the test is negative for the presence of coliforms, the water is considered acceptable for human consumption.

McFeters *et al.* (1995) compared the performance of Colisure™, a selective differential medium incorporating 4-MU-GAL, with LTB in a MPN assay. The study looked at the detection of low numbers of coliform bacteria and *E. coli* in samples mimicking contaminated drinking water containing chlorine. The authors summarised that Colisure™ had excellent sensitivity and specificity for the detection of coliforms

and *E. coli* and that the indicator organisms were recovered with greater efficiency using Colisure™ as no confirmatory tests were required.

These testing systems have many advantages over traditional techniques, such as: confirmatory tests are not required; inoculation and interpretation is straightforward; simultaneous detection of *E. coli* and total coliforms is possible in the same sample. While an evaluation sponsored by the US EPA comparing the performance of: i) the Colilert system; ii) Standard Methods MPN; iii) a quantitative test (Edberg *et al.*, 1988b, 1991); and iv) the qualitative P-A test (Edberg *et al.*, 1989, 1991) recognised these advantages, it also recognised certain limitations of the Colilert system. These include: injured coliforms can give a weaker yellow colour making interpretation difficult; the Colilert test has proved unreliable in the presence of high numbers of background heterotrophs. The detection of β-galactosidase-positive *Aeromonas* and *Flavobacterium* species giving false-positive results is particularly concerning (Edberg *et al.*, 1988b; Covert *et al.*, 1989; Tryland and Fiksdal, 1998). A small number of the flavobacteria and *Bacteroides* spp. have also been found to produce β-glucuronidase (Frampton and Restaino, 1993). Several commonly occuring freshwater plants have been shown to interfere in bacterial ß-galactosidase or ß-glucuronidase assay (Davies et al., 1994), as has the influence of seawater (Pommepuy et al., 1996). In addition to commercial preparations, chromogens and fluorogens can be incorporated directly into a variety of other media to enumerate total coliforms and *E. coli* using MF (Berg and Fiksdal, 1988; Covert *et al.*, 1989; Mates and Shaffer, 1989; Sartory and Howard, 1992; Brenner *et al.*, 1993; Grant, 1997).

Luminescent ß-galactosidase substrates have been synthesised (Geiger *et al.*, 1992; Arakawa *et al.*, 1998) which have been incorporated into a bioluminescence assay (Masuda-Nishimura *et al.*, 2000). The assay involves the hydrolysis of D-luciferin-*O*-ß-galactopyranoside (LuGal) by coliform bacteria in a P-A test. Observations made after 7 h of culture followed by a 10 min enzyme assay using LuGAL were comparable to those made after a 22 - 24 h culture a fluorescent assay using 4-MU-GAL. The authors suggested that the detection limit for a luminescent substrate was approximately 50-fold more sensitive that a test using a fluorogenic substrate.

**Direct impedance technology**

There is an increasing interest in the use of impedance for the rapid detection of coliforms and *E. coli* in water samples. Impedance is the apparent resistance in an electrical circuit to the flow of alternating current. When micro-organisms grow in a culture medium, they metabolise substrates of lower conductivity to products of higher conductivity and thereby decrease the impedance of the medium. The concept of impedance-based measurement of microbial growth was originally developed in 1899 by Stewart, but it was nearly 80 years later before it was used for microbial water assessment (Jay, 1991), showing that the technique is capable of detecting as few as 10-100 cells. Owens *et al.* (1985) reported that *E. coli* will give a large conductance change in growth medium containing trimethylamine N-oxide (TMAO): the conversion of TMAO to trimethylamine (TMA) only occurs in the presence of fermentable carbohydrate (Ogden and Cann, 1987). The use of TMAO with D-glucuronic acid as the fermentable carbohydrate has been investigated (Ogden, 1993), with the intention of making the medium specific for *E. coli*.

A comparative study investigating the above medium in conjunction with Colilert (Colquhoun *et al.*, 1995) showed TMAO/D-glucuronic acid to have good specificity for *E. coli* when screened against a range of different bacteria. Similar performances were obtained when this method was compared both with MF methods and Colilert, suggesting that this method may be suitable for the routine examination of potable water, although coliforms and *E. coli* cannot be detected simultaneously (Colquhoun *et al.*, 1995). However, the enzyme ß-glucuronidase is not responsible for the fermentation of D-glucuronic acid, and so the presence of this carbohydrate in a medium would not make it specific for *E. coli*. The 'specificity' this literature is referring to is most likely due to the different end-products generated by the fermentation of sugars by different organisms. For example, *E. coli* produces end-products of a low pH when fermenting glucose, whereas *Klebsiella* sp. and *Enterobacter* sp. generate end-points of a more neutral pH (acetylmethylcarbinol or 'acetoin'). This difference in fermentation pathways is demonstrated by the use of the MR test (methyl red) for the differentiation of *E. coli* from *Klebsiella* sp. and *Enterobacter* sp. when fermenting glucose, and the VP test (Voges-Proskauer) for distinguishing *Klebsiella* sp. from *E. coli*, when fermenting glucose (MacFaddin, 1976).

**PCR: polymerase chain reaction-based detection systems**

The polymerase chain reaction (PCR) technique simulates *in vitro* the DNA replication process that occurs *in vivo* and can result in millions of copies of the target DNA sequence being created (Gleeson and Gray, 1997). Bej *et al.* (1990) were first to develop a genetic procedure for the detection of coliforms and *E. coli*. The method

involves the extraction of DNA, amplification of target nucleotide sequences specifically associated with coliforms (by PCR) and subsequent detection of the amplified DNA using 'gene probes'. Gene probes are small pieces of labelled nucleic acid that hybridise with a homologous complementary probe sequence in the target micro-organism (Bej *et al.*, 1991). Used alone, the probes are not sensitive enough to detect micro-organisms present in small numbers, but when combined with PCR, they become highly sensitive in detecting low numbers of organisms. The sequences chosen by Bej *et al.*, (1990) to be amplified by PCR were a region of the *lac Z* gene (Fricker and Fricker, 1994), encoding for β-galactosidase, and a region of *mal B*, which codes for maltose transport. The *lam B* gene was also amplified, which encodes a surface protein specific for *E. coli*. Therefore, amplification of *lac Z* would occur for all coliforms, while amplification of *lam B* would be specific for *E. coli*. (Gleeson and Gray, 1997). Further developments of this procedure amplified the region of the *E. coli* genome encoding for the β-glucuronidase gene, *uid A*, specifically detecting *E. coli* and *Shigella* spp., including β-glucuronidase-negative strains of *E. coli* which generally go undetected using other techniques as they do not express enzyme activity (Bej *et al.*, 1991; Iqbal *et al.*, 1997).

A major limitation for the routine use of PCR is the equipment and expertise required as well as the use of radioisotopes, which smaller laboratories may lack the facilities to dispose of safely. Non-radiolabelled PCR detection methods are now available, but there are still many problems associated with PCR in environmental monitoring. PCR technology has been applied to the detection of a wide range of micro-organisms in a variety of applications and, while it does not appear to be practicable to apply the technology directly with some sample types, it offers the possibility markedly to reduce

the time taken to obtain a result (Fricker and Fricker, 1994). With a growing emphasis on molecular biology and associated techniques, PCR and the use of multiplex gene probes (where multiple primers are used to amplify multiple target sequences so that more than one microbial pathogen can be detected in a single water sample: Lang *et al.*, 1994), appear to have significant potential in environmental monitoring (Geldreich, 1992).

**Immunodiagnostic techniques**

Recent years have seen a large expansion in the number of immunoassays available for the detection of coliforms and *E. coli*, such as agglutination, radio-immuno assays, enzyme-linked immuno-sorbent assays (ELISA), immuno-fluorescent techniques, immuno-enzyme assays, monoclonal antibodies and counter-immunoelectrophoresis. The use of these techniques is largely for the identification and enumeration of specific pathogens in water, as opposed to the routine analysis of water (Kfir *et al.*, 1993). Monoclonal antibodies have been utilised to detect coliforms and *E. coli* (Joret *et al.*, 1989): this approach was found to be very specific, allowing rapid detection of these indicators. However the inability of monoclonal antibody techniques to distinguish between viable and non-viable cells means that they cannot be used for routine water analysis.

# Aims

The four principal aims of the study were:

1) To evaluate several novel chromogenic ß-galactosidase substrates for quantitative analysis of coliforms in membrane filtration assay.
2) To develop and evaluate a novel dual substrate medium, incorporating two different coloured chromogens for ß-galactosidase and ß-glucuronidase, for the simultaneous detection of total coliforms and *E. coli* by membrane filtration.
3) To investigate the properties of a range of novel fluorogenic coumarin derivatives, in terms of their relative fluorescence, toxicity and suitability for use in a broth-based assay for ß-galactosidase and/or ß-glucuronidase activity.
4) To develop and evaluate a novel, rapid broth-based MPN assay for coliforms using a fluorogenic ß-galactosidase substrate.

# CHAPTER TWO

## The evaluation of chromogenic substrates for the detection of ß-galactosidase activity in coliforms

# Background

There are many media available for the enumeration of total coliforms and faecal coliforms by membrane filtration. Most media employed in public health microbiology laboratories contain selective agents in order to reduce the growth of non-target organisms (Sartory and Watkins, 1999). At present m-ENDO is the medium recommended in US standard methods (Anon., 2000) and membrane lauryl sulphate broth (MLSB) is recommended in UK standard methods (Anon., 1994). Since the development of the first selective medium for *E. coli* (MacConkey, 1908) the incorporation of bile salts or synthetic detergent alternatives such as Teepol 610 (Jameson and Emberley, 1956) and sodium lauryl sulphate (Stanfield and Irving, 1981) have been the primary selective agents in media for the enumeration of coliforms from water. Anaerobic incubation has been examined as a selective pressure for the inhibition of non-target aerobic bacteria but reduced recovery rates and increased costs indicated traditional selective media were superior (Shirey and Bissonnette, 1997). The selective nature of these media requires them to suppress unwanted background flora (e.g. Gram-positive organisms) and prevent the swarming of *Proteus* species. Although suitable for the isolation of coliforms from faeces, these agents have been shown to be more inhibitory to environmentally-damaged or oxidatively-stressed coliforms (Anon., 1980; McFeters *et al.*, 1986). Selective media, such as m-ENDO agar and MLSB, have been ranked amongst the lowest in a survey of the relative effectiveness of over 20 media commonly used for coliform analysis, when using injured coliforms (McFeters *et al.*, 1982). Sodium deoxycholate, an ingredient of m-ENDO agar has been found to interfere with flagellation and motility of *E. coli*, as well as the uptake of

some carbohydrates (D'Mello and Yotis, 1987). The use of less harsh constituents and enzyme-specific substrates allows significant improvements in the identification and enumeration of target bacteria (Sartory and Watkins, 1999).

The enzyme ß-galactosidase catalyses the breakdown of lactose into galactose and glucose and therefore is mostly used for enumerating the coliform group, since they possess this enzyme (Manafi *et al.*, 1991). Precise enumeration of coliforms is achievable with media employing a substrate for ß-galactosidase that yields an easily-detected product without reliance on other enzymes in the lactose fermentation pathway (Sartory and Watkins, 1999). This is due to the fact that atypical coliform bacteria exist which lack lactose permease, a second enzyme necessary for the uptake and successful fermentation of lactose by a cell, and traditional methodologies would fail to detect these bacteria as coliforms.

Many chromogenic substrates have been described for the demonstration of bacterial enzymes (Killian and Bülow, 1976; Manafi *et al.*, 1991; Dealler, 1993; James, 1994; Ciebin, 1995). The action of a particular bacterial enzyme on a specific substrate is demonstrated by the liberation of a strongly-coloured product. In contrast to the use of pH indicators to detect the products of fermentation, chromogenic substrates do not depend on the presence of metabolic pathways but only a single enzyme (Dealler, 1993). Those substrates designed to specifically detect ß-galactosidase by the release of a coloured aglycone that remains localised on bacterial colonies are of particular value in diagnostic microbiology (James *et al.*, 2000a, 2000b). This is because they may be successfully applied in agar-based media and they enable visualisation of target colonies within a mixed culture. The commercially available indoxylic substrate

X-GAL (5-bromo-4-chloro-3-indolyl-ß-D-galactoside) is particularly suited to such a role (Ley *et al.*, 1988) and has been applied in a number of chromogenic culture media (e.g. Rambach, 1990; Poupart *et al.*, 1991).

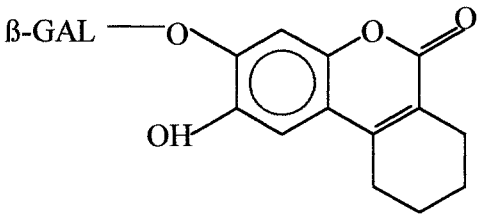
Some chromogenic substrates, known as simultaneous capture chromogenic substrates (Dealler, 1993), incorporate metal ions upon hydrolysis to produce coloured chelates that are highly insoluble and therefore remain localised around the bacterial colony. This is particularly relevant as one potential problem when using a chromogenic substrate in an agar-based medium is diffusion of the aglycone throughout the medium, making it difficult to distinguish between positive and negative colonies in a mixed culture. This approach has been successfully used in the design of glycosides based on 8-hydroxyquinoline (James and Yeoman, 1988), 3,4-cyclohexenoesculetin (James *et al.*, 1997) and alizarin (James *et al.*, 2000a). Such substrates have been successfully employed in chromogenic media (Larinkari and Rautio, 1995; Perry *et al.*, 1999).

Four novel chromogenic β-D-galactoside substrates, three of which are simultaneous capture chromogenic substrates, were evaluated and are described below, along with the commercially available substrate X-GAL which was also investigated in this study:

i) 3,4-Cyclohexenoesculetin-β-D-galactoside (CHE-GAL), which incorporates the use of ferric ions ($Fe^{3+}$) for visualisation (see Figure 2.1). The aglycone released by hydrolysis forms a black complex with ferric ions to give a clearly visible, non-diffusible product.

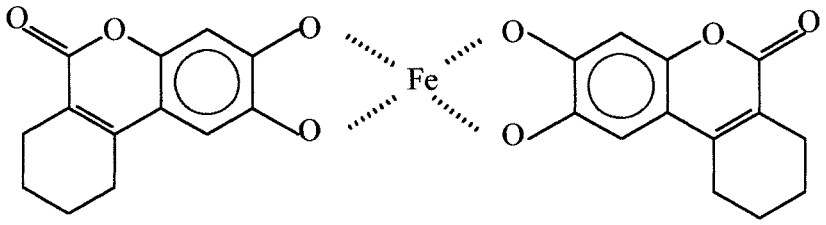
**Figure 2.1: Structure of 3,4-cyclohexenoesculetin-β-D-galactoside (CHE-GAL) and the complex formed following hydrolysis. (a) CHE-GAL; (b) cyclohexenoesculetin molecules released by hydrolysis associate with iron to form a black insoluble complex.**

a)





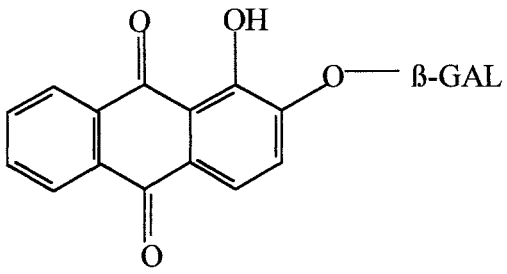

b)



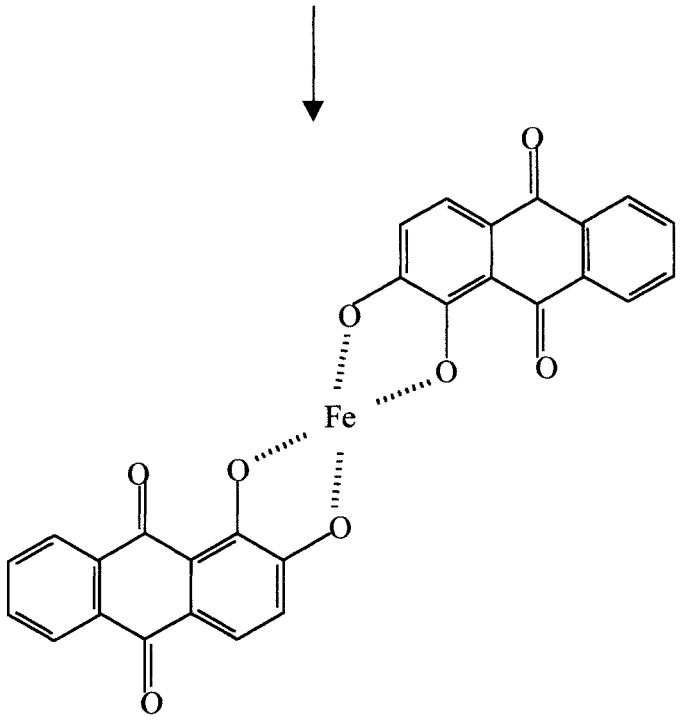

ii) Alizarin-β-D-galactoside (ALIZ-GAL), which operates in a similar way to CHE-GAL (see Figure 2.2). This compound is known to chelate a variety of metal ions, forming brightly-coloured dyes (James *et al.*, 2000a). The chelates are formed in the same way regardless of which metal ion is used but the colour of the precipitate that localises around the bacterial colony varies. This substrate was used in this study with aluminium ions ($Al^{3+}$) and ferric ions ($Fe^{3+}$), to form pink and purple precipitates respectively.

**Figure 2.2: Structure of alizarin-β-D-galactoside (ALIZ-GAL) and the complex formed following hydrolysis. (a) ALIZ-GAL; (b) the complex formed by the released alizarin with iron .**

a)

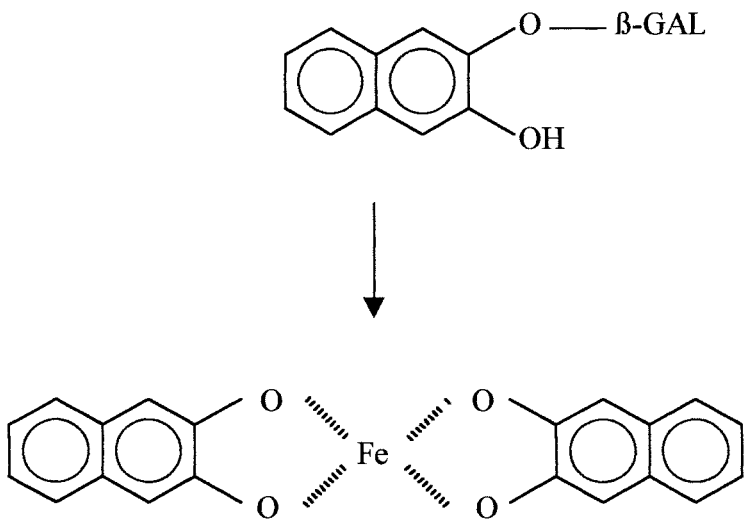



b)




iii) Dihydroxynaphthalene-β-D-galactoside (DHN-GAL), which is cleaved by β-galactosidase to release dihydroxynaphthalene which also forms metal chelates with ferric ions (see Figure 2.3). ß-galactosidase-positive colonies appear purple upon hydrolysis of this substrate.

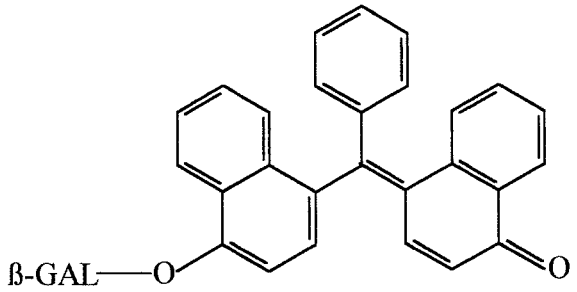
**Figure 2.3: Structure of dihydroxynaphthalene-ß-D-galactoside (DHN-GAL) and the complex formed following hydrolysis. (a) DHN-GAL; (b) the complex formed by the released dihydroxynaphthalene with iron.**




iv) *p*-naphtholbenzein-ß-D-galactoside (PNB-GAL), which does not form a chelate upon hydrolysis (see Figure 2.4) and so has the advantage of not requiring the addition of a metal salt to the medium. The *p*-naphtholbenzein released by hydrolysis is a highly coloured molecule which remains localised on bacterial colonies which consequently appear pink (James *et al.*, 2000b). The fact that this substrate does not require the addition of a metal salt can be an advantage as deaminase activity may also generate coloured products when a medium incorporates both peptone and iron (Manafi and Rotter, 1991). Phenylalanine deaminase has been used for the specific recognition of strains of *Proteus* spp. (Singer and Volcani, 1955). The enzyme is present in all *Proteus* spp., *Providencia* spp., *Moraxella phenylpyruvica* and a few *Pseudomonas* spp. (Bascomb, 1987). Detection of activity by conventional methods is based on reaction of the keto

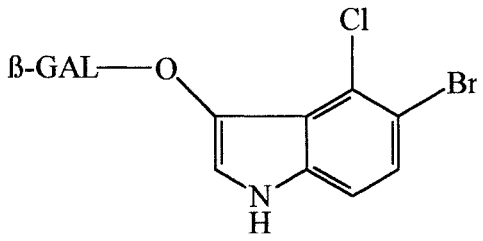
group, formed by deamination of phenylalanine or tryptophan, with ferric chloride ($FeCl_3$) in acid conditions to give a green colour (Singer and Volcani, 1955). However, this enzyme has shown activity with a variety of amino acids (Pelmont *et al.*, 1972). Therefore when a medium contains peptones, which will consist of a wide range of amino acids, and ferric ions there is the possibility that certain species will form coloured colonies leading to a high number of false-positives.

**Figure 2.4 Structure of *p*-naphtholbenzein-ß-D-galactoside (PNB-GAL)**




v) Indoxylic galactosides such as indoxyl-β-galactoside and halogenated derivatives including X-GAL are commercially available and highly effective substrates for bacterial ß-galactosidase detection (see Figure 2.5). They have the advantage when used in solid media that the aglycone released is oxidised rapidly by air to produce an insoluble coloured product which is restricted to the colony mass (Kodaka *et al.*, 1995). ß-galactosidase-positive colonies appear bright blue when X-GAL is incorporated in the growth medium, while ß-galactosidase-negative colonies appear colourless.

**Figure 2.5 Structure of 5-bromo-4-chloro-3-indolyl-ß-D-galactoside (X-GAL)**




Among the various stresses to which Enterobacteriaceae are submitted when discharged into aquatic systems, solar radiation is one of the most important in reducing coliform numbers in natural waters (Kapuscinski and Mitchell, 1981; Davies and Evison, 1991, Davies-Colley *et al.*, 1994). Both solar UV-A (320-400 nm) and UV-B (280-320 nm) have been shown to be responsible for bacterial inactivation (Calkin and Barcelo, 1982), although other studies have reported that faecal coliform inactivation may result from UV-A only, e.g. at wavelengths of light greater than 370 nm (Kapuscinski and Mitchell, 1983). In the present study, UV-A has been used as a means of creating a population of damaged coliforms, to see whether such organisms can be enumerated on media containing novel chromogenic substrates, in comparison with the US standard, non-chromogenic coliform medium.

# Experimental objectives

1) To evaluate the relative effectiveness of several novel chromogens in comparison with commercially available X-GAL with a wide range of coliforms.
2) To investigate performance of novel chromogens compared to US standard m-ENDO medium in a membrane filtration format.
3) To evaluate the use of novel chromogens for the enumeration of UV-A damaged organisms.

# Materials and methods

## Bacterial strains

Bacterial strains were obtained from National Collection of Type Cultures (NCTC, Central Public Health Laboratory, London, UK) or National Collection of Industrial and Marine Bacteria Limited (NCIMB, Aberdeen, UK). Wild strains were isolated from pathological samples in the Microbiology Department, Freeman Hospital, Newcastle upon Tyne.

## Growth media

For the purpose of this study m-ENDO was made up from its constituents rather than the pre-mixed formulation available commercially so that, when necessary, lactose and basic fuchsin could be omitted. Yeast extract, tryptone, peptone P, tryptose, dipotassium phosphate, monopotassium phosphate, sodium chloride, lactose, basic fuchsin, sodium deoxycholate, sodium lauryl sulphate and bacteriological agar (No. 1) were all obtained from BDH (Poole, UK). Columbia agar was obtained from LabM (Bury, UK)

## Substrates and chemicals

CHE-GAL, ALIZ-GAL, PNB-GAL, DHN-GAL were kindly synthesised by Dr. A. L. James. X-GAL was obtained from Glycosynth (Warrington, UK). Isopropyl-ß-D-

thiogalactoside (IPTG), Ringer's solution tablets (¼ strength), ferric ammonium citrate and potassium aluminium sulphate were obtained from Sigma-Aldrich Chemicals (Poole, UK).

**Equipment**

All media were prepared using a Sartorius 2434 electronic balance; accurate to 0.1 mg (Sartorious Limited, Epsom, UK); the pH of each medium was checked using a pH meter (Hanna Instruments Limited, Leighton Buzzard, UK). A membrane filtration system consisting of a vacuum pump, manifold, six magnetic funnels and cellulose ester membrane filters (pore size 0.45 μm) diameter 47 mm were used throughout this investigation (Gelman Sciences, Ann Arbor, USA). Small volumes were dispensed using calibrated Gilson semi-automatic pipettes (P200 and P1000) with sterile disposable tips (Gilsen Medical Electronics, Villiers-le-Bel, France). Large volumes were dispensed using sterile disposable 10 ml pipettes (L.I.P. Limited, Shipley, UK). All organisms were prepared to a specific suspension density using a Densimat (bioMérieux, La Balme-les-Grottes, France). A multipoint inoculator (Denley) was used for experiments involving the optimisation of substrate concentration. All plates were incubated in a LEEC 37°C incubator (Laoratory and Electrical Engineering Company, Nottingham, UK). A UV-A cabinet (two Bellarium SA1-12 UV-A fluorescent tubes, each of 80 W power output) was used for the simulation of environmentally-stressed organisms. The UV-A irradiance was 18.5 W $m^{-2}$, measured directly at the bench surface (Skye UV-A sensor, Llandrindod Wells, UK). Any biochemical confirmation of strains was carried out using Analytical Profile Index (API) 20 E strips and associated software (bioMérieux, La Balme-les-Grottes, France).

**Evaluation of chromogenic substrates for enumeration of coliforms in membrane filtration format.**

Prior to their use in a membrane filtration assay, it was necessary to establish an appropriate concentration of each of the chromogenic substrates. This has been documented for four of the chromogenic galactosides in this study, namely CHE-GAL (300 mg $l^{-1}$), ALIZ-GAL (50 mg $l^{-1}$), PNB-GAL (100 mg $l^{-1}$) and X-GAL (80 mg $l^{-1}$), as detailed by Perry *et al.,* 1999, James *et al.*, 2000a, James *et al.*, 2000b and Ley *et al.*, 1993 respectively. DHN-GAL was incorporated into media at the same concentration as CHE-GAL (300 mg $l^{-1}$) as both substrates form chelated coloured precipitates in the same way; ferric ions ($Fe^{3+}$) were also included at the same concentration (as ferric ammonium citrate, at 500 mg $l^{-1}$) in both media. All of the chromogenic galactosides used in this study were added to media prior to autoclaving, since it has been demonstrated that the addition of X-GAL prior to autoclaving had no detectable impact on its performance (James *et al.*, 2000b).

A total of 8 media formulations were evaluated, 7 of which incorporated the use of m-ENDO base formulation. Columbia agar was included as a non-selective growth control. Four strains of each of the following coliforms (including one or more NCTC strains of each coliform where possible) were membrane filtered in duplicate onto 8 different m-ENDO-based agar media: *Citrobacter freundii* (NCTC 9750, FRHCFR1, FRHCFR2, FRHCFR3); *Enterobacter cloacae* (NCTC 11936, FRHECL7, FRHECL8, FRHECL9); *Escherichia coli* (NCTC 10418, FRHECO1, FRHECO2, FRHECO3); *Hafnia alvei* (FRHHAL1, FRHHAL2, FRHHAL3, FRHHAL4); *Klebsiella pneumoniae* (NCTC 10896, FRHKPN9 FRHKPN10, FRHKPN11);

*Serratia marcescens* (NCTC 10211), and *Serratia* spp. (FRHSEX1, FRHSEX2, FRHSEX3); *Yersinia enterocolitica* (NCTC strains 11176, 10938, 10463, 10461). Each organism was membrane filtered in duplicate for each modification of m-ENDO agar medium. To limit the number of plates required, two filter membranes were cultured per plate, duplicate samples were processed on different plates ensuring fair replicate data. m-ENDO base formulation was as follows, with all components in g $l^{-1}$: yeast extract, 1.2; tryptone, 3.7; peptone P, 3.7; tryptose, 7.5; dipotassium hydrogen phosphate, 3.3; potassium dihydrogen phosphate, 1.0; sodium chloride, 3.7; sodium sulphite, 1.6*

(* excluded from final formulation, see later).

Sufficient m-ENDO base to make 2 litres of media, was weighed and dissolved fully by heating to 50°C in a water bath. Each of the substrates were weighed carefully into separate 250 ml Duran bottles, along with the required amount of bacteriological agar for each 250 ml volume of medium, as detailed on the following page:

| | | /250 ml |
|---|---|---|
| Duran 1 | ALIZ-GAL | 12.5 mg |
| | ferric ammonium citrate | 125 mg |
| | bacteriological agar | 2.5 g |
| Duran 2 | ALIZ-GAL | 12.5 mg |
| | potassium aluminium sulphate | 125 mg |
| | bacteriological agar | 2.5 g |
| Duran 3 | CHE-GAL | 75 mg |
| | ferric ammonium citrate | 125 mg |
| | bacteriological agar | 2.5 g |
| Duran 4 | PNB-GAL | 25 mg |
| | bacteriological agar | 2.5 g |
| Duran 5 | DHN-GAL | 75 mg |
| | ferric ammonium citrate | 125 mg |
| | bacteriological agar | 2.5 g |
| Duran 6 | X-GAL | 20 mg |
| | bacteriological agar | 2.5 g |
| Duran 7 | lactose | 2.35 g |
| (m-ENDO) | basic fuchsin | 0.2 g |
| | bacteriological agar | 2.5 g |
| Duran 8 | columbia agar | 10.25 g |

To each Duran bottle 225 ml of m-ENDO base was added, excluding Duran bottle 8 (Columbia agar control) to which 250 ml of water was added at this stage, all bottles were mixed thoroughly. Each medium was sterilised by autoclaving at 116°C for 10 minutes and cooled gently to 50°C. The selective agents of m-ENDO agar cannot be autoclaved and so a single stock solution was prepared to contain both selective agents at 10 x strength: sodium deoxycholate (10 x = 1 g $l^{-1}$) and sodium lauryl sulphate (10 x = 0.5 g $l^{-1}$). Both agents were added to hot (80-90°C) sterile distilled water, gently cooled and the stock solution was stored at 5°C. Aliquots of 25 ml of stock solution were added aseptically to each Duran bottle (excluding Duran 8, Columbia agar control) after sterilisation and cooling to 50°C.

After addition of the stock solution a 2 ml sample was removed from each medium to allow the pH of the medium to be checked and the whole volume was then adjusted to pH 7.2 ± 0.2 if necessary. Once poured, a test plate of each medium was inoculated with *E. coli* (NCTC 10418) for overnight incubation (37°C); all remaining plates were incubated at 37°C overnight as a sterility check, prior to use. Fresh overnight cultures grown on Columbia blood agar were suspended in aliquots of ¼ strength Ringer's solution (2 ml) in plastic Bijoux, to reach a density equivalent to a MacFarland standard of 2.0 (≈ 6 x $10^8$ CFU/ml). Suspensions were diluted 1:1000, then again 1:1000, followed by 1:50 to attain 100 ml volumes of an estimated 12 CFU/ml, of which 5 ml was filtered to achieve an estimated 60 CFU/membrane. All dilutions/filtrations were carried out with ¼ strength sterile Ringer's solution.

To 5 sterile magnetic funnels, approximately 20 ml of ¼ strength Ringer's solution was added, followed by 5 ml of organism suspension using a sterile 10 ml disposable

pipette. Each magnetic funnel was labelled appropriately so that 5 organisms could be membrane-filtered onto all media before it was necessary to re-sterilise the funnels. Membranes were placed carefully onto each agar medium with sterile forceps. Each funnel was then treated in a boiling water bath to prevent carry-over and allowed to cool before use with a subsequent sample. Plates were incubated for 18 h at 37°C.

**Investigation into anomalies observed with PNB-GAL and X-GAL when incorporated into m-ENDO base.**

It was noted from test plates that both PNB-GAL and X-GAL exhibited colourless colonies with coliforms known to be ß-galactosidase-positive. Subsequent tests showed that this did not occur when the substrates were incorporated into Columbia agar base. In addition, PNB-GAL showed some background colouring of the medium when incorporated into a Columbia agar base, but this did not occur in m-ENDO base medium, where the medium was colourless/transparent. It appeared that a component of the m-ENDO base formulation was inhibiting the formation of the coloured precipitate formed upon hydrolysis of these substrates.

To investigate which component was causing the problem a solution of the coloured product of PNB-GAL, *p*-naphtholbenzein, was prepared. This solution incorporated the buffers required for m-ENDO base (dipotassium hydrogen phosphate 3.3 g $l^{-1}$; potassium dihydrogen phosphate 1.0 g $l^{-1}$). The solution was heated to 56°C and split into 8 aliquots of 5 ml in sterile glass test-tubes. To each test-tube one of the following components was added, which collectively made up m-ENDO base. The tubes were held at 56°C and observed for decolorization of the product.

| | | g $l^{-1}$ | amount added (mg) |
|---|---|---|---|
| Tube 1: | yeast extract | 1.2 | 6.0 |
| Tube 2: | tryptone | 3.7 | 18.5 |
| Tube 3: | peptone P | 3.7 | 18.5 |
| Tube 4: | sodium chloride | 3.7 | 18.5 |
| Tube 5: | sodium desoxycholate | 0.1 | 0.5 |
| Tube 6: | sodium lauryl sulphate | 0.05 | 0.25 |
| Tube 7: | sodium sulphite | 1.6 | 8.0 |
| Tube 8: | control | - | - |

Any medium component which resulted in decolorization of the coloured product was noted and confirmation was carried out by the preparation of both PNB-GAL agar and X-GAL agar (in m-ENDO base), with and without the presence of the particular component. Plates were inoculated with NCTC type strains of *E. coli* (NCTC 10418: ß-galactosidase positive) and *S. typhimurium* (NCTC 74: ß-galactosidase negative); the same *E. coli* was also membrane filtered, and all plates were incubated for 18 h at 37°C.

**Membrane filtration of UV-A damaged organisms**

The membrane filtration investigation of chromogenic substrates was repeated following UV-A exposure of a selection of strains from the original study. These included wild strains of *E. coli* (FRHECO2 and FRHECO3), *K. pneumoniae* (FRHKPN9 and FRHKPN10) and *C. freundii* (FRHCFR2 and FRHCFR3). Organisms were suspended in sterile distilled water and exposed to UV-A light for 30 minutes to

reduce the overall counts on non-selective medium (Columbia agar). It is also likely that some of the remaining bacteria will have been sub-lethally damaged by UV-A exposure, and that such damage may increase their sensitivity to selective agents and inhibitory components such as those used in m-ENDO medium. Such sub-lethal damage may also result in increased toxicity of chromogens and this was evaluated by comparing the counts for the various chromogenic media used in the present study. All plates were counted after 18 h incubation at 37oC.

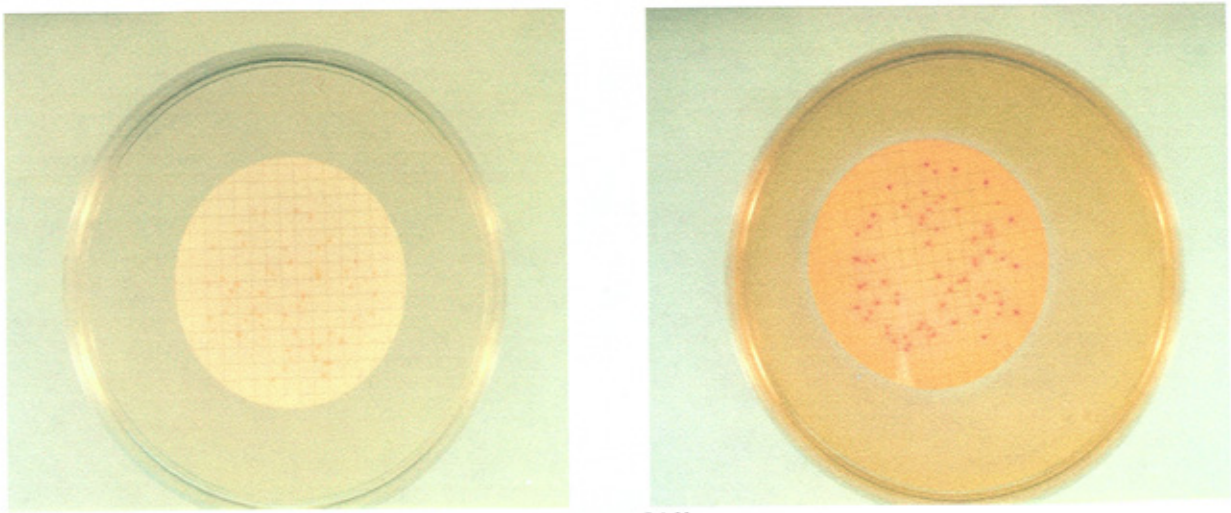
# Results and discussion

## Investigation into anomalies observed with PNB-GAL and X-GAL when incorporated into m-ENDO base.

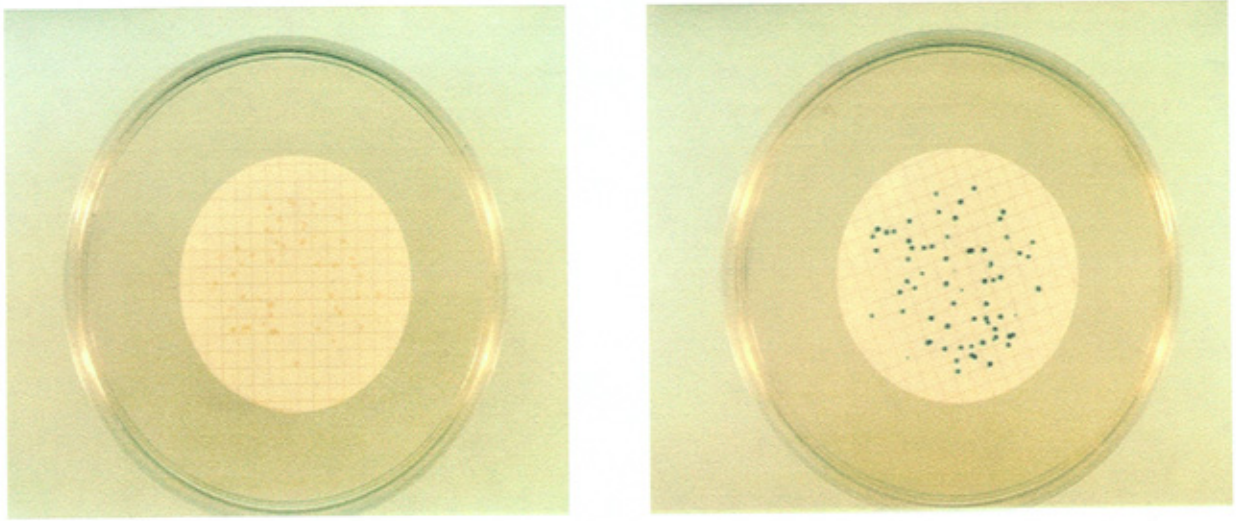
Sodium sulphite was confirmed as inhibiting formation of the coloured product when present in both X-GAL and PNB-GAL media formulations. Within 7 minutes, the tube containing this component plus the relevant core molecule became completely colourless while all other components of m-ENDO medium had no effect on the colour of the core molecules. Confirmation plates of X-GAL and PNB-GAL that included sodium sulphite in the m-ENDO base formulation exhibited colourless *E. coli* and *S. typhimurium* colonies after 18 h incubation. However, growth did not appear to be affected (see Figure 2.6a and 2.6b for *E. coli*). As *E. coli* is ß-galactosidase positive the colonies should have appeared blue and pink respectively. Sodium sulphite is a powerful reducing agent used to create anaerobic conditions with a low redox. The indoxyl released upon hydrolysis of X-GAL appears colourless when in the presence of sodium sulphite as indoxylic substrates rely on oxidation to form coloured precipitates. However, PNB-GAL is a substrate which does not rely on oxidation to form coloured precipitates; it is therefore unclear why the products of PNB-GAL hydrolysis appeared colourless in the presence of sodium sulphite.

**Figure 2.6: Photograph of a) PNB-GAL, and b) X-GAL in m-ENDO base i) with and ii) without the presence of sodium sulphite (organism: *E. coli* NCTC 10418).**

**a)i** **a)ii**



**b)i** **b)ii**



One of the reasons sodium sulphite is present in m-ENDO is to reduce basic fuchsin, the pH indicator present in the medium. It has been observed that basic fuchsin is inhibitory to certain strains of *E. coli* when used without sodium sulphite (Rungpetch Khaengraeng, personal communication). This could explain the slight inhibition observed with the m-ENDO media formulations used in this research. In this study, as it was necessary to use the same m-ENDO base formulation for all media, it was decided that sodium sulphite should be excluded from work involving the use of m-ENDO base. With hindsight, an alternative approach would have been to include two variations of m-ENDO, one with sodium sulphite and one without, to more fully evaluate the effect of sodium sulphite.

**Evaluation of chromogenic substrates for enumeration of coliforms in membrane filtration format.**

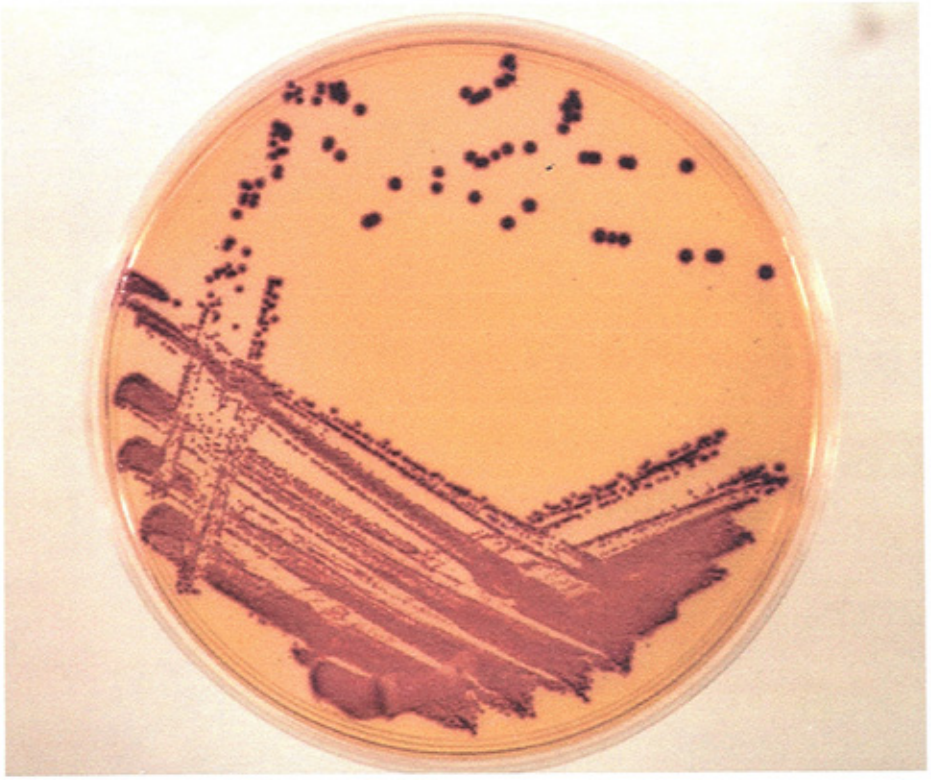
Figures 2.7 - 2.11 show the coloured colonies formed upon hydrolysis of some of the chromogenic substrates by ß-galactosidase positive organisms. James *et al.* (1996) described the synthesis of a new chromogenic substrate for the detection of ß-galactosidase (CHE-GAL), where the substrate was incorporated into Columbia agar at a concentration of 500 mg $l^{-1}$. A more recent publication (Perry *et al.*, 1999) described a new chromogenic medium for the isolation of *Salmonella* spp. that incorporated both CHE-GAL (300 mg $l^{-1}$) and X-α-GAL (80 mg $l^{-1}$) and this concentrations of substrate was used in the present study. Figure 2.7 shows that the m-ENDO base medium incorporating CHE-GAL exhibited no background colour and generated an intense black precipitate upon hydrolysis, which remained localised to the bacterial colonies.

**Figure 2.7: Photograph of CHE-GAL in Columbia agar with iron showing streak dilution of *E. coli* (NCTC 10418).**

James et al. (2000a) described the substrate alizarin-ß-D-galactoside (ALIZ-GAL) and suggested that it should be incorporated in media at 50 mg $l^{-1}$ and this concentration was used in the present study. Figures 2.8 and 2.9 show the coloured colonies formed upon hydrolysis of ALIZ-GAL with ferric ions (Figure 2.8a and b) and aluminium ions (Figure 2.9a and b). The photographs indicate that a slight coloration of the background medium was observed with the inclusion of these substrates, but that this did not affect the visualisation of single colonies. Figures 2.8b and 2.9b show in close-up the lack of diffusion of the coloured precipitate around each ß-galactosidase-positive colony.

**Figure 2.8: Photograph of ALIZ-GAL in Columbia agar with iron showing a) streak dilution of *E. coli* (NCTC 10418); b) close-up of single colonies illustrating localisation of coloured precipitate around bacterial colony.**

**a)**



**b)**

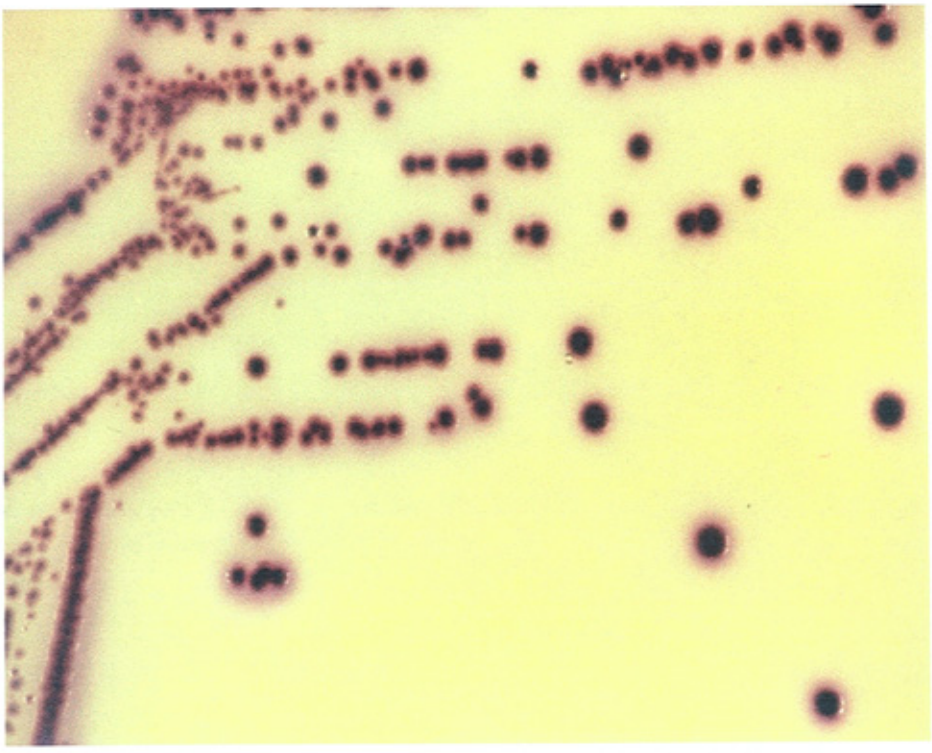
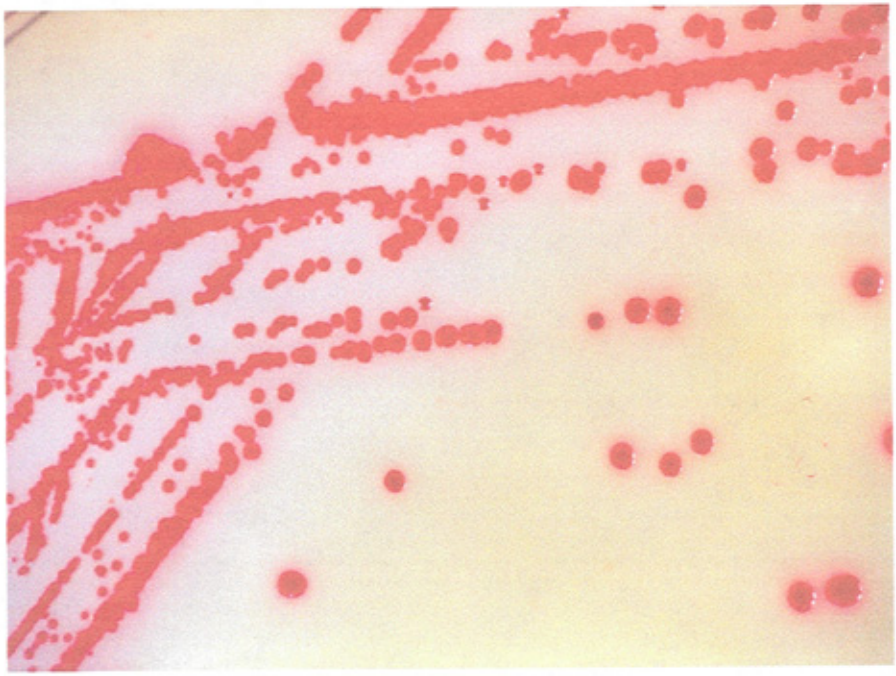


**Figure 2.9: Photograph of ALIZ-GAL in Columbia agar with aluminium showing a) streak dilution of *E. coli* (NCTC 10418); b) close-up of single colonies illustrating localisation of coloured precipitate around bacterial colony.**

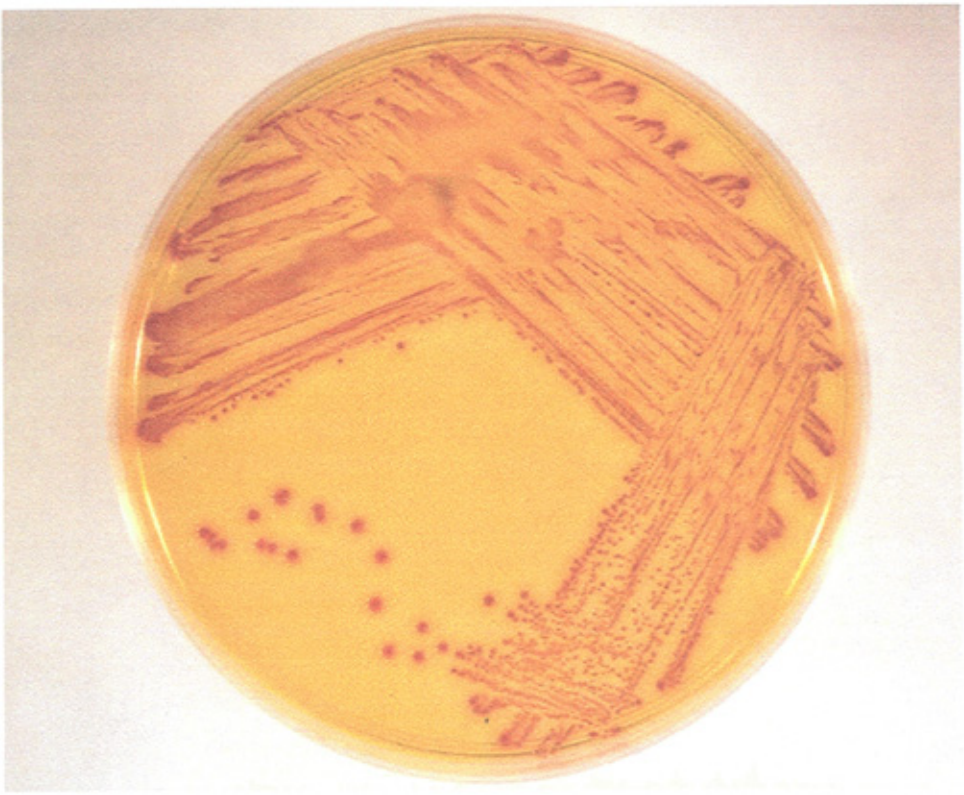
**a)**

**b)**



James *et al.* (2000b) evaluated PNB-GAL for incorporation into media to detect ß-galactosidase. The substrate was most sensitive when at a concentration of 100 mg $l^{-1}$ and this value was used in the present study. The inclusion of PNB-GAL in the medium generated a slight background coloration but again this did not adversely affect the performance of the medium, as shown in Figure 2.10. Colonies that were ß-galactosidase-positive were observed as deep pink in colour, with little diffusion.

**Figure 2.10: Photograph of PNB-GAL in Columbia agar showing a streak dilution of *E. coli* (NCTC 10418).**

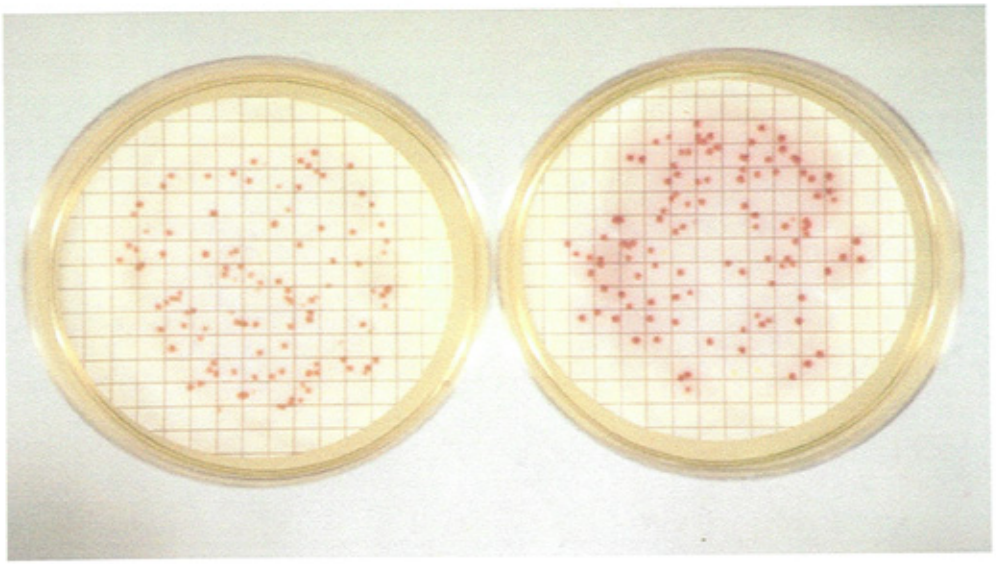


Ley *et al.* (1993) compared a new medium mX-GAL to m-ENDO agar using membrane filtration of raw and treated water supplies. mX-GAL medium contained similar ingredients to m-ENDO (with the exclusion of sodium sulphite, tryptose, tryptone and basic fuchsin) and incorporated X-GAL at a concentration of 100 mg $l^{-1}$. Figure 2.11 illustrates the blue colonies exhibited by the hydrolysis of X-GAL in the presence of a ß-galactosidase-positive organism. Once again, there is no diffusion of the coloured molecule around positive colonies making them easily distinguishable.

**Figure 2.11: Photograph of X-GAL in Columbia agar showing a streak dilution of *E. coli* (NCTC 10418).**

DHN-GAL was used at an equivalent concentration to CHE-GAL (300 mg $l^{-1}$), as explained in the Materials and Methods. This DHN-GAL concentration proved to be effective, as colonies were bright purple with little or no diffusion and background colour was minimal, as shown in Figure 2.12.

**Figure 2.12: Photograph of DHN-GAL in Columbia agar with and without IPTG showing a membrane filter with colonies of *E. coli* (NCTC 10418).**



Although the hydrolysis of all the chromogenic substrates by ß-galactosidase producers generated coloured colonies with little diffusion, some appeared to have more potential than others for incorporation into a chromogenic medium. ALIZ-GAL offered a strong advantage over other substrates because it can be used at low concentrations and can form differently coloured colonies depending on the metal ions present. Furthermore, the colour generated was very intense, whereas the colour generated by the hydrolysis of PNB-GAL and DHN-GAL was not as intense. Columbia agar and PNB-GAL exhibited a slightly orange background colour, possibly an indication that this substrate is not stable and is beginning to auto-hydrolyse. In summary, the most promising substrates at this stage appeared to be CHE-GAL, ALIZ-GAL and X-GAL.

The data in Table 2.1 shows the average total colony count (i.e. for all β-galactosidase-positive and β-galactosidase-negative colonies) for each strain on each medium (taken from Appendix 2.1). The data illustrate that the counts were higher than anticipated for all strains tested, based on the calculations set out in the Materials and Methods, and they were especially high for *Serratia* sp. However, all of the test organisms were still countable on the membrane filters. The high counts for *Serratia* sp. results in an increased mean value on all of the test media (Table 2.1).

Paired t tests were carried out to compare the data for total counts on non-selective Columbia agar against those on the various selective media. The t test was used in one-sided format, to see whether the potentially inhibitory components present in the various selective media resulted in counts that were, on average, significantly lower than those on the non-selective medium. The t values and (one-sided) P values in Table 2.1 suggest that all of the chromogenic media included in this study exhibited mean total colony counts that were not statistically significantly lower than those obtained on Columbia agar medium, since all P values obtained were greater than 0.05 (the Excel® output is shown in Appendix 2.2). In contrast to the chromogenic media, the mean total colony count exhibited on m-ENDO medium gave a P value of 0.026, indicating a statistically significantly lower colony count compared to the growth control (Columbia agar medium). This inhibitory effect could be related, in part, to the omission of sodium sulphite from the basal m-ENDO media formulation, as detailed in the Materials and Methods.

**Table 2.1: Average total count of coliforms on chromogenic media, m-ENDO and Columbia agar.**

| Organism | ALIZ-GAL ($Fe^{3+}$) | ALIZ-GAL ($Al^{3+}$) | CHE-GAL ($Fe^{3+}$) | PNB-GAL | X-GAL | DHN-GAL ($Fe^{3+}$) | m-ENDO | Columbia |
|---|---|---|---|---|---|---|---|---|
| *E. coli* | | | | | | | | |
| NCTC 10418 | 83 | 32 | 128 | 84 | 17 | 120 | 0 | 155 |
| FRHECO1 | 71 | 73 | 59 | 57 | 52 | 35 | 47 | 64 |
| FRHECO2 | 111 | 93 | 100 | 90 | 96 | 68 | 92 | 85 |
| FRHECO3 | 173 | 179 | 157 | 133 | 142 | 156 | 156 | 143 |
| *E. cloacae* | | | | | | | | |
| NCTC 11936 | 165 | 158 | 165 | 172 | 173 | 159 | 138 | 198 |
| FRHECL7 | 154 | 156 | 150 | 157 | 136 | 138 | 145 | 147 |
| FRHECL8 | 143 | 134 | 149 | 107 | 158 | 101 | 161 | 138 |
| FRHECL9 | 190 | 144 | 163 | 181 | 181 | 161 | 190 | 198 |
| *H. alvei* | | | | | | | | |
| FRHHAL2 | 137 | 140 | 143 | 123 | 97 | 127 | 90 | 144 |
| FRHHAL3 | 351 | 265 | 198 | 200 | 194 | 201 | 206 | 205 |
| FRHHAL4 | 194 | 122 | 117 | 167 | 175 | 126 | 153 | 158 |
| FRHHAL5 | 79 | 89 | 79 | 78 | 75 | 67 | 72 | 91 |
| *C. freundii* | | | | | | | | |
| NCTC 9750 | 109 | 109 | 117 | 107 | 103 | 91 | 90 | 98 |
| FRHCFR1 | 164 | 145 | 147 | 147 | 128 | 158 | 156 | 126 |
| FRHCFR2 | 87 | 80 | 92 | 85 | 79 | 76 | 84 | 72 |
| FRHCFR3 | 95 | 95 | 91 | 90 | 97 | 89 | 95 | 79 |
| *K. pneumoniae* | | | | | | | | |
| NCTC 10896 | 0 | 1 | 37 | 0 | 87 | 0 | 1 | 100 |
| FRHKPN9 | 125 | 128 | 121 | 115 | 114 | 123 | 113 | 117 |
| FRHKPN10 | 61 | 65 | 66 | 61 | 64 | 70 | 57 | 68 |
| FRHKPN11 | 66 | 76 | 68 | 69 | 77 | 68 | 67 | 66 |
| *S. marcescens* | | | | | | | | |
| NCTC 10211 | 290 | 267 | 336 | 324 | 318 | 374 | 352 | 317 |
| *Serratia* sp. | | | | | | | | |
| FRHSEX1 | 242 | 221 | 252 | 292 | 252 | 302 | 196 | 294 |
| FRHSEX2 | 280 | 295 | 271 | 289 | 266 | 280 | 232 | 255 |
| FRHSEX3 | 311 | 317 | 294 | 310 | 311 | 305 | 294 | 289 |
| *Y. enterocolitica* | | | | | | | | |
| NCTC 11176 | 97 | 111 | 112 | 120 | 120 | 91 | 119 | 128 |
| NCTC 10938 | 81 | 79 | 83 | 72 | 76 | 78 | 75 | 69 |
| NCTC 10463 | 75 | 73 | 83 | 83 | 76 | 91 | 77 | 87 |
| NCTC 10461 | 112 | 91 | 115 | 107 | 108 | 111 | 90 | 112 |
| Mean | 144 | 133 | 139 | 136 | 134 | 134 | 127 | 143 |
| P value | 0.427 | 0.117 | 0.172 | 0.104 | 0.081 | 0.071 | 0.026 | - |
| T value | -0.187 | 1.215 | 0.960 | 1.287 | 1.438 | 1.512 | 2.034 | - |

Table 2.2 shows the data from Table 2.1, converted to percentage growth for each of the coliform strains, when compared to Columbia agar. These data highlight that some organisms struggled to grow on certain test media, in particular *E. coli* NCTC 10418 and *K. pneumoniae* NCTC 10896. This table also includes the overall mean value for the percentage growth indicating that, in general, growth was most inhibited on m-ENDO and was least inhibited on CHE-GAL. However, none of the test media were completely non-inhibitory to coliform growth.

**Table 2.2: Percentage growth of coliforms on chromogenic media and m-ENDO when compared to growth on Columbia agar.**

| Organism | ALIZ-GAL ($Fe^{3+}$) | ALIZ-GAL ($Al^{3+}$) | CHE-GAL ($Fe^{3+}$) | PNB-GAL | X-GAL | DHN-GAL ($Fe^{3+}$) | m-ENDO | Columbia |
|---|---|---|---|---|---|---|---|---|
| *E. coli* | | | | | | | | |
| NCTC 10418 | 53 | 20 | 82 | 54 | 11 | 77 | NG | 100 |
| FRHECO1 | 100 | 100 | 91 | 88 | 80 | 55 | 73 | 100 |
| FRHECO2 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 |
| FRHECO3 | 100 | 100 | 100 | 93 | 99 | 100 | 100 | 100 |
| *E. cloacae* | | | | | | | | |
| NCTC 11936 | 83 | 80 | 83 | 87 | 87 | 80 | 70 | 100 |
| FRHECL7 | 100 | 100 | 100 | 100 | 92 | 94 | 99 | 100 |
| FRHECL8 | 100 | 97 | 100 | 78 | 100 | 73 | 100 | 100 |
| FRHECL9 | 96 | 73 | 82 | 91 | 91 | 81 | 96 | 100 |
| *H. alvei* | | | | | | | | |
| FRHHAL2 | 95 | 97 | 99 | 85 | 67 | 88 | 62 | 100 |
| FRHHAL3 | 100 | 100 | 97 | 98 | 94 | 98 | 100 | 100 |
| FRHHAL4 | 100 | 77 | 74 | 100 | 100 | 80 | 97 | 100 |
| FRHHAL5 | 87 | 98 | 87 | 86 | 83 | 74 | 79 | 100 |
| *C. freundii* | | | | | | | | |
| NCTC 9750 | 100 | 100 | 100 | 100 | 100 | 92 | 92 | 100 |
| FRHCFR1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| FRHCFR2 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| FRHCFR3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *K. pneumoniae* | | | | | | | | |
| NCTC 10896 | NG | 1 | 37 | NG | 87 | NG | 1 | 100 |
| FRHKPN9 | 100 | 100 | 100 | 98 | 97 | 100 | 97 | 100 |
| FRHKPN10 | 90 | 96 | 98 | 90 | 95 | 100 | 84 | 100 |
| FRHKPN11 | 99 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *S. marcescens* | | | | | | | | |
| NCTC 10211 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Serratia* sp. | | | | | | | | |
| FRHSEX1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| FRHSEX2 | 100 | 100 | 100 | 100 | 100 | 100 | 91 | 100 |
| FRHSEX3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Y. enterocolitica* | | | | | | | | |
| NCTC 11176 | 76 | 87 | 87 | 94 | 94 | 71 | 93 | 100 |
| NCTC 10938 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| NCTC 10463 | 86 | 83 | 95 | 95 | 87 | 100 | 89 | 100 |
| NCTC 10461 | 100 | 82 | 100 | 96 | 96 | 100 | 80 | 100 |
| Mean | 92% | 89% | 93% | 91% | 92% | 87% | 86% | 100% |

Table 2.3 shows the average count for ß-galactosidase-positive colonies of each strain on the six chromogenic media and on m-ENDO (derived from the data shown in Appendix 2.1). Comparisons of the difference between each chromogenic medium and m-ENDO medium using a one-sided paired t-test of the same format as that described previously for the total count data gave the t values and P values listed in Table 2.3 (the Excel® output is shown in Appendix 2.2). In this instance, the one-sided t-test was used to determine whether the result for the selective medium was significantly higher than that for m-ENDO medium. The data showed that all chromogenic media included in this study gave mean positive counts that were statistically significantly higher than those observed on m-ENDO, since the P values were all substantially less than 0.05. Columbia agar counts were omitted from this particular data analysis as they represent total colony counts on a non-selective medium and so cannot be included when comparing ß-galactosidase-positive colony counts between selective media used for the enumeration of coliforms. The results give an indication that the number of positive colonies observed when using this formulation of the recommended US standard coliform medium (m-ENDO) was somewhat lower that those obtained when using the tested chromogenic media.

**Table 2.3: Average count of ß-galactosidase-positive coliforms on chromogenic media and m-ENDO.**

| Organism | ALIZ-GAL ($Fe^{3+}$) | ALIZ-GAL ($Al^{3+}$) | CHE-GAL ($Fe^{3+}$) | PNB-GAL | X-GAL | DHN-GAL ($Fe^{3+}$) | m-ENDO | Columbia |
|---|---|---|---|---|---|---|---|---|
| *E. coli* | | | | | | | | |
| NCTC 10418 | 83 | 32 | 128 | 84 | 17 | 120 | 0 | - |
| FRHECO1 | 71 | 73 | 59 | 57 | 52 | 35 | 47 | - |
| FRHECO2 | 111 | 93 | 100 | 90 | 96 | 68 | 0 | - |
| FRHECO3 | 173 | 179 | 157 | 133 | 142 | 156 | 156 | - |
| *E. cloacae* | | | | | | | | |
| NCTC 11936 | 165 | 158 | 165 | 172 | 173 | 159 | 138 | - |
| FRHECL7 | 154 | 156 | 150 | 157 | 136 | 138 | 145 | - |
| FRHECL8 | 143 | 134 | 149 | 107 | 158 | 101 | 0 | - |
| FRHECL9 | 190 | 144 | 163 | 181 | 181 | 161 | 190 | - |
| *H. alvei* | | | | | | | | |
| FRHHAL2 | 105 | 106 | 106 | 123 | 97 | 127 | 90 | - |
| FRHHAL3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| FRHHAL4 | 194 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| FRHHAL5 | 79 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| *C. freundii* | | | | | | | | |
| NCTC 9750 | 109 | 109 | 117 | 107 | 103 | 91 | 0 | - |
| FRHCFR1 | 164 | 145 | 147 | 147 | 128 | 158 | 156 | - |
| FRHCFR2 | 87 | 80 | 92 | 85 | 79 | 76 | 84 | - |
| FRHCFR3 | 95 | 95 | 91 | 90 | 97 | 89 | 95 | - |
| *K. pneumoniae* | | | | | | | | |
| NCTC 10896 | 0 | 0 | 37 | 0 | 87 | 0 | 0 | - |
| FRHKPN9 | 125 | 128 | 121 | 115 | 114 | 123 | 113 | - |
| FRHKPN10 | 61 | 65 | 66 | 61 | 64 | 70 | 57 | - |
| FRHKPN11 | 66 | 76 | 68 | 69 | 77 | 68 | 67 | - |
| *S. marcescens* | | | | | | | | |
| NCTC 10211 | 290 | 267 | 336 | 324 | 318 | 374 | 0 | - |
| *Serratia* sp. | | | | | | | | |
| FRHSEX1 | 242 | 221 | 252 | 292 | 252 | 302 | 0 | - |
| FRHSEX2 | 280 | 295 | 271 | 289 | 266 | 280 | 0 | - |
| FRHSEX3 | 311 | 317 | 294 | 310 | 311 | 305 | 0 | - |
| *Y. enterocolitica* | | | | | | | | |
| NCTC 11176 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| NCTC 10938 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| NCTC 10463 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| NCTC 10461 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| Mean | 118 | 102 | 109 | 107 | 105 | 107 | 48 | - |
| P value | 0.0006 | 0.004 | 0.002 | 0.004 | 0.004 | 0.005 | - | - |
| T value | -3.642 | -2.910 | -3.122 | -2.90 | -2.91 | -2.780 | - | - |

Table 2.4 illustrates the same data set, expressed as the percentage of ß-galactosidase-positive coliforms when compared to the average total count (based on the data contained in Appendix 2.1). As in other data sets, Table 2.4 includes the mean percentage value for each of the test media. These data clearly indicates that not only was m-ENDO the most inhibitory to bacterial growth, it was also the most inhibitory to ß-galactosidase expression. An anomaly observed with these data was that all strains of *Y. enterocolitica* included in the study did not express ß-galactosidase activity on any of the test media, and three of the four *H. alvei* strains expressed ß-galactosidase activity on ALIZ-GAL + $Fe^{3+}$ medium only. *Hafnia* sp. are observed to be very weak producers of ß-galactosidase activity in standard laboratory tests (Dr. J. D. Perry, personal communication), and this could be due either to limited expression of the ß-galactosidase gene or to an absence of lactose permease, which would mean that it would takes longer for the substrate to enter bacterial cells.

**Table 2.4: Percentage of ß-galactosidase-positive coliforms on chromogenic media and m-ENDO when compared to averaged total coliform count.**

| Organism | ALIZ-GAL ($Fe^{3+}$) | ALIZ-GAL ($Al^{3+}$) | CHE-GAL ($Fe^{3+}$) | PNB-GAL | X-GAL | DHN-GAL ($Fe^{3+}$) | m-ENDO | Columbia |
|---|---|---|---|---|---|---|---|---|
| *E. coli* | | | | | | | | |
| NCTC 10418 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | - |
| FRHECO1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| FRHECO2 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | - |
| FRHECO3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| *E. cloacae* | | | | | | | | |
| NCTC 11936 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| FRHECL7 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| FRHECL8 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | - |
| FRHECL9 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| *H. alvei* | | | | | | | | |
| FRHHAL2 | 77 | 75 | 74 | 100 | 100 | 100 | 100 | - |
| FRHHAL3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| FRHHAL4 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| FRHHAL5 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| *C. freundii* | | | | | | | | |
| NCTC 9750 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | - |
| FRHCFR1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| FRHCFR2 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| FRHCFR3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| *K. pneumoniae* | | | | | | | | |
| NCTC 10896 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | - |
| FRHKPN9 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| FRHKPN10 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| FRHKPN11 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| *S. marcescens* | | | | | | | | |
| NCTC 10211 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | - |
| *Serratia* sp. | | | | | | | | |
| FRHSEX1 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | - |
| FRHSEX2 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | - |
| FRHSEX3 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | - |
| *Y. enterocolitica* | | | | | | | | |
| NCTC 11176 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| NCTC 10938 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| NCTC 10463 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| NCTC 10461 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| Mean | 78% | 71% | 74% | 71% | 75% | 71% | 43% | - |

**Enumeration of UV-A damaged organisms by membrane filtration**

A standard plate count (viable count) was carried out by preparing serial decimal dilutions of test bacteria both before and after UV-A exposure, followed by culture onto Columbia agar for 18 h at 37oC (Table 2.5). The average plate count for all 6 test organisms post UV-A exposure (1.31 x $10^6$ CFU/ml) was 69% of the pre-exposure TVC value (1.92 x $10^6$ CFU/ml). Therefore bacterial numbers were reduced by approximately one-third after 30 min exposure to UV-A. Although such reductions in counts were not substantial, it is reasonable to assume that a proportion of the remaining organisms would have been damaged by the UV-A exposure and that such damage might well then affect their ability to recover on selective media.

**Table 2.5: Viable plate counts of six coliform strains pre and post 30 min UV-A exposure.**

| Organism | Pre-UV-A exposure (CFU $ml^{-1}$) | Post-UV-A exposure (CFU $ml^{-1}$) |
|---|---|---|
| *E. coli* | | |
| (FRHECO2) | 1.5 x $10^6$ | 1.38 x $10^6$ |
| (FRHECO3) | 2.2 x $10^6$ | 1.59 x $10^6$ |
| *K. pneumoniae* | | |
| (FRHKPN9) | 1.3 x $10^6$ | 1.1 x $10^6$ |
| (FRHKPN10) | 2.6 x $10^6$ | 1.42 x $10^6$ |
| *C. freundii* | | |
| (FRHCFR2) | 1.7 x $10^6$ | 1.2 x $10^6$ |
| (FRHCFR3) | 2.2 x $10^6$ | 1.19 x $10^6$ |

Table 2.6 shows the averaged number of total colonies observed on each test medium after UV-A exposure (taken from Appendix 2.3). This number represents both the total number of colonies and the number of ß-galactosidase-positive colonies, since all of the colonies observed on the test media were ß-galactosidase-positive. This is in agreement with the data for these coliform strains without exposure to UV-A (c.f. Table 2.1 and 2.3), with the exception of *E. coli* (FRHECO2) for which none of the 92 colonies which grew on m-ENDO exhibited ß-galactosidase activity. This could indicate that m-ENDO can exhibit differences with the same strain under different physiological states, resulting in false data. This anomaly did not occur on any of the chromogenic media.

**Table 2.6: Average total count of UV-A damaged (ß-galactosidase-positive) coliforms on chromogenic media, m-ENDO and Columbia agar.**

| Organism | ALIZ-GAL ($Fe^{3+}$) | ALIZ-GAL ($Al^{3+}$) | CHE-GAL ($Fe^{3+}$) | PNB-GAL | X-GAL | DHN-GAL ($Fe^{3+}$) | m-ENDO | Columbia |
|---|---|---|---|---|---|---|---|---|
| *E. coli* | | | | | | | | |
| FRHECO2 | 129 | 135 | 130 | 146 | 162 | 135 | 125 | 159 |
| FRHECO3 | 48 | 55 | 52 | 61 | 52 | 68 | 58 | 181 |
| *C. freundii* | | | | | | | | |
| FRHCFR2 | 144 | 162 | 180 | 184 | 150 | 172 | 148 | 208 |
| FRHCFR3 | 134 | 116 | 139 | 110 | 137 | 146 | 116 | 160 |
| *K. pneumoniae* | | | | | | | | |
| FRHKPN9 | 152 | 166 | 182 | 164 | 146 | 172 | 136 | 176 |
| FRHKPN10 | 188 | 173 | 184 | 190 | 156 | 172 | 165 | 140 |
| Mean | 132 | 134 | 144 | 142 | 134 | 144 | 125 | 171 |

Table 2.7 shows the data expressed as a percentage of the counts observed on Columbia agar. The data indicate that, in general, the most inhibitory medium was m-ENDO, as was observed earlier for non-UV-A treated strains (Table 2.4). All chromogenic media performed similarly, with an average colony count 79% of the value of the non-selective control medium while the percentage for m-ENDO was lowest, at 72%.

**Table 2.7: Percentage growth of UV-A damaged (ß-galactosidase-positive) coliforms on chromogenic media and m-ENDO when compared to growth on Columbia agar.**

| Organism | ALIZ-GAL ($Fe^{3+}$) | ALIZ-GAL ($Al^{3+}$) | CHE-GAL ($Fe^{3+}$) | PNB-GAL | X-GAL | DHN-GAL ($Fe^{3+}$) | m-ENDO | Columbia |
|---|---|---|---|---|---|---|---|---|
| *E. coli* | | | | | | | | |
| FRHECO2 | 81 | 85 | 81 | 92 | 100 | 85 | 79 | 100 |
| FRHECO3 | 26 | 30 | 29 | 34 | 29 | 38 | 32 | 100 |
| *C. freundii* | | | | | | | | |
| FRHCFR2 | 69 | 78 | 87 | 88 | 72 | 83 | 71 | 100 |
| FRHCFR3 | 84 | 73 | 87 | 69 | 86 | 89 | 73 | 100 |
| *K. pneumoniae* | | | | | | | | |
| FRHKPN9 | 86 | 94 | 100 | 93 | 83 | 98 | 77 | 100 |
| FRHKPN10 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Mean | 75% | 77% | 81% | 79% | 79% | 82% | 72% | 100% |

Taken together, the results of the present study show that all of the chromogenic media included in the study were less-inhibitory than the m-ENDO formulation, exhibiting higher total colony counts by up to 12%, though never as high as for the non-selective medium (Columbia agar). The number of ß-galactosidase-positive colonies observed was also substantially higher for all chromogenic media when compared to m-ENDO. Some strains were more inhibited than others on the majority of test media, namely *E. coli* NCTC 10418 and *K. pneumoniae* NCTC 10896 (Table 2.2). This may indicate that the selective components of m-ENDO base (sodium deoxycholate, sodium lauryl sulphate and basic fuchsin, in the case of m-ENDO agar) were inhibiting the growth of some members of the coliform group and suppressing the enzymatic activity of certain organisms.

Niemi *et al.*, (2001) observed that different membrane filtration media varied in their yield of coliform bacteria. The authors compared three membrane filtration media (m-ENDO, differential coliform agar and lactose Tergitol 7 TTC agar) for the isolation of coliforms from well waters. The authors observed that 92% of typical colonies isolated on m-ENDO medium belonged to the Enterobacteriaceae, in comparison to 74% and 75% on differential coliform agar and TTC agar respectively. However, differential coliform agar yielded the widest range of species, whereas m-ENDO yielded more *Serratia* sp. than any other test media. The observations of Niemi *et al.* suggest that m-ENDO is less inhibitory to the Enterobacteriaceae than other membrane filtration media, these results are contrary to those observed in this study, where m-ENDO was the most inhibitory of all the test media. However, it is possible that this is due to the modified formulation of m-ENDO (sodium sulphite exclusion).

The performance of all the novel chromogenic substrates was good; some substrates were highly sensitive at low concentrations and compared favourably with X-GAL, which has been applied in a number of chromogenic media (Rambach, 1990; Poupart *et al.*, 1991). The results indicate some highly effective alternatives to X-GAL, for example: the effective concentration of ALIZ-GAL (50 mg $l^{-1}$) is just over half that of X-GAL (80 mg $l^{-1}$). Furthermore, the synthesis of these substrates is relatively straightforward and produces a high yield of product, particularly for PNB-GAL, as the core molecule required for derivitisation is available commercially and is inexpensive (James *et al.*, 2000b). The ability of alizarin to form differently coloured chelates with various cations is a further advantage and this lends itself to the development of a dual substrate chromogenic medium. However, a significant advantage when using the cyclohexenoesculetin-based glycosides is that the precipitate formed is black in colour and remains highly localised to the bacterial colony. Therefore when it is used to detect very small numbers of indicator organisms in a highly mixed population it is comparatively easy to detect a positive colony. A further advantage of all of the novel chromogenic substrates is that they can be applied to anaerobic systems, whereas X-GAL can only be used aerobically as the formation of colour is dependent on oxidation (Kodaka *et al.*, 1995).

In conclusion, the information in this Chapter gives an indication that if chromogenic media were used routinely in water analysis systems with an m-ENDO-based medium formulation a more accurate representation of the number of coliforms present in a water sample may be achieved. When using chromogenic media the laborious process of colony counting is generally easier since a deeply coloured precipitate is formed, as opposed to the formation of colour due to the effects of a change in pH on a pH

indicator dye, which tends to diffuse around the colony, making heavily inoculated plates particularly difficult to read. Such chromogenic substrates would also be an advantage when dealing with large sample numbers, where it becomes more difficult to distinguish clearly between positive and negative colonies using conventional substrates.

# CHAPTER THREE

## Application of chromogenic substrates to detect both coliforms and *Escherichia coli* using a single membrane filtration assay system

# Background

The studies with the novel chromogenic substrates for ß-galactosidase described in Chapter 2 have shown these compounds to be both stable and less inhibitory to coliform growth than the recommended medium in US standard methods. All of the chromogenic substrates tested generated intensely-coloured colonies following hydrolysis of the substrate by the bacterial enzyme. The development of a membrane filtration technique based on these compounds may provide an improved means of detecting and enumerating coliforms, compared to conventional media. The most appropriate combination would be a ß-galactosidase substrate, to detect any coliform organisms present in a sample, with a ß-glucuronidase substrate, to detect the presence of *E. coli.* A prerequisite of this combination would be that the colour formed from the hydrolysis of the ß-galactoside could be masked by the colour formed from the hydrolysis of the ß-glucuronide. This is so that *E. coli*, which possesses both of these enzymes would be observed as ß-glucuronidase-positive, so that this faecal indicator bacterium would be detected in the presence of other coliforms.

The enumeration of *E. coli* in water samples has traditionally relied upon detection of lactose-fermenting colonies in a selective medium at 44°C, followed by demonstration of indole production at the same temperature (Anon., 1994; Anon., 2000). One disadvantage of this methodology is that isolated colonies must be regarded as presumptive *E. coli* until a subsequent overnight indole test can be performed. Also the method fails to detect non-lactose-fermenting strains of *E. coli* as well as those which

fail to grow at 44°C. As a result of work focusing on ß-glucuronidase as a marker for the detection of *E. coli*, it is now apparent that this enzyme is largely restricted to *E. coli*, although 40-67% of *Shigella* spp. and 17-29% *Salmonella* spp. also have the enzyme (Frampton and Restaino, 1993), 90-97% of strains of *E. coli* produce ß-glucuronidase in readily detectable amounts (Feng and Hartman, 1982). Consequently the detection of ß-glucuronidase activity is used increasingly for the identification of *E. coli* from water samples and has become an approved method in the United States (Anon., 2000).

A specific novel substrate was synthesised for this study, which allows the direct demonstration of ß-glucuronidase activity by *E. coli* colonies. The substrate incorporates the same core molecule as a ß-D-galactoside substrate previously described (James *et al.*, 1996), i.e. cyclohexenoesculetin (CHE). Hydrolysis of this new substrate for ß-glucuronidase activity, cyclohexenoesculetin-ß-D-glucuronide (CHE-GUR), yields a black, non-diffusible product which remains totally restricted to the bacterial colony (as described earlier, see Figure 2.1). The development of this chromogenic substrate is of particular interest because of the colour formed upon hydrolysis by the bacterial enzyme. The likelihood of environmental coliforms being present in river water samples is much higher than the likelihood of faecal coliforms being present. The hydrolysis of a chromogenic substrate used to detect environmental coliforms could therefore result in an abundance of coloured colonies present on a membrane filter. With this newly developed chromogenic substrate for the most abundant faecal coliform (i.e. ß-glucuronidase-expressing *E. coli*), any ß-glucuronidase-positive colonies present (*E. coli*) will be clearly visible, as they will be black in colour. The second advantage of this novel substrate is that, regardless of

the chromogen used for ß-galactosidase detection, *E. coli* colonies will be visualised due to the black precipitate formed by the chelating substrate upon hydrolysis, masking any colour produced from the chromogenic substrate for ß-galactosidase.

For each water sample to be tested using UK standard methods, duplicate samples must be membrane filtered onto the test media, both of which are incubated at 30°C for 4 hours, after which one membrane is transferred to 37°C and the other to 44°C for a further 14 hours incubation (Anon., 1994). The substrate combination described in the introductory paragraph would have the added advantage of not requiring duplicate samples to be prepared for two different split incubation temperatures as only one incubation temperature of 37°C would be required, reducing costs significantly. The membrane filtration method used in this study employed X-GAL (see Figure 2.5 and Figure 2.11) and CHE-GUR, as described above, in a modified formulation of membrane lauryl sulphate broth (MLSB, Anon., 1994), which is the standard medium used in the UK. In this approach water samples were filtered onto a single membrane filter which was then placed on an absorbent pad containing the chromogenic medium. After incubation for 4 hours at 30°C followed by 14 hours at 37°C the number of blue and black colonies could be counted, constituting the number of presumptive total coliforms and presumptive *E. coli* in the water sample.

Another glycoside substrate was included in this investigation, to establish whether it could reduce false-positive coliform identifications. X-α-GAL is commercially available and is an analogous chromogenic substrate to X-GAL with the exception that the sugar molecule is attached to the chromogen in an α-1-4-linkage, rather than a ß-1-4-linkage, so that only organisms possessing the α-galactosidase enzyme would be able

to hydrolyse the substrate and generate colour. Previous work indicates that most coliforms which possess ß-galactosidase also possess α-galactosidase, although they may not be strong producers of the latter (Perry et al., 1999). One of the main causes of false-positive presumptive coliform results is the presence of oxidase-positive bacteria capable of fermenting lactose (Hussong *et al.*, 1981). In some instances up to 14% of presumptive coliforms counted using membrane filtration can be false-positive (Mates and Shaffer, 1989); for example, *Aeromonas* spp. are able to produce acid and gas from lactose at 37°C and are therefore regarded as presumptive coliforms until they are excluded by subsequent negative confirmatory tests.

*Aeromonas* spp. have been isolated from both chlorinated and un-chlorinated drinking water supplies (Burke *et al.*, 1984); they have also been isolated in waters containing no *E. coli* and few total coliforms (Schbert, 1991). *Aeromonas hydrophila* has been found to be the most commonly identified species, with frequencies ranging from 40% to 58%, in a study of organisms isolated from various water sources (Grabow and Du Preez, 1979). *Aeromonas* spp. make up part of the Vibrionaceae family and can be defined as oxidase-positive, facultatively anaerobic, Gram-negative rods. If *Aeromonas* spp. possess the ß-galactosidase enzyme but not the α-galactosidase enzyme, then the inclusion of X-α-GAL would eliminate false-positive results from *Aeromonas* spp., due to the fact that colonies would remain colourless on the membrane filter.

An additional document is to be included in the new edition of 'The Bacteriological Examination of Drinking Water Supplies' (UK standard methods) describing cultural techniques for the comparison of methods for drinking water bacteriology, a draft version of this protocol has recently been published (Anon., 2001). The document

provides a protocol for comparing the recoveries of confirmed target organisms by two methods, but is also applicable to comparison of more than two methods. This draft protocol was followed in order to provide a valid method comparison using simulated contaminated water samples in this investigation. A number of source waters can be used in this protocol: the present study used sewage samples from various treatment stages and river water samples to vary the indigenous flora present, in order to evaluate the novel chromogenic media described above against the UK standard method. All source waters were manipulated to produce chlorine-stressed samples, so that the comparative data are relevant to coliforms and *E. coli* in 'real' drinking waters.

## Experimental objectives

1) To evaluate the performance of a novel chromogenic medium containing X-GAL and CHE-GUR, designed to simultaneously detect coliforms and *E. coli* in a direct comparison with MLSB, the medium recommended in UK guidelines (Anon., 1994).
2) To assess the potential of a commercially-available chromogenic α-galactoside substrate in detecting coliforms.

# Materials and methods

## Growth media

For the purpose of this study membrane lauryl sulphate broth (MLSB) was made up from its constituents rather than the pre-mixed formulation available commercially so that, when necessary, lactose and phenol red could be omitted. Peptone, yeast extract, lactose, and phenol red were all obtained from BDH (Poole, UK). Sodium lauryl sulphate (>99% pure) was obtained from Sigma-Aldrich Chemicals (Poole, UK). Lactose peptone water (LPW) and tryptone water (TW) were made up from their constituents also. Tryptone and sodium chloride were obtained from BDH (Poole, UK).

## Substrates, chemicals and equipment

CHE-GUR was synthesised, while X-GAL and X-α-GAL were obtained from Glycosynth (Warrington, UK). The chemicals required for chlorine damage and analysis were as follows, and were all obtained from Sigma-Aldrich Chemicals: sodium hypochlorite solution (≈15% w/v active chlorine); N,N-diethyl-p-phenylenediamine (DPD); ferrous ammonium sulphate (FAS); sulphuric acid; phosphoric acid; barium diphenylamine indicator; potassium dichromate; potassium iodide. Diagnostic reagents such as oxidase reagent (N,N,N,N-tetramethyl-*p*-phenylenediamine) and the constituents of Kovac's reagent (*p*-dimethylaminobenzaldehyde, isoamyl alcohol, hydrochloric acid) were obtained from Sigma-Aldrich Chemicals. Kovac's reagent was

made up as described by MacFaddin (1976). All equipment used is described fully in Chapter two.

**Protocol for the generation of chlorine-stressed organisms using river water and sewage samples as the source of target organisms**

A 10 litre volume of laboratory tap water was collected in a suitable container and a one litre volume of river water was also collected in a Duran bottle. Both were cooled and stored at 4 – 8°C until required. Using guidelines supplied from Analytical Environmental Services (Tyne and Wear, UK) the amount of free and total chlorine in the cooled tap water only was measured: from this value the amount of chlorine to be added was calculated so that a final free chlorine concentration of 0.1 – 0.5 mg/l was achieved (see Appendix 3.1). The exact amount of chlorine required to achieve this concentration depended on a number of factors including pH, organic matter content and inorganic matter content of the water. Chlorine was added in the form of a sodium hypochlorite solution. The laboratory tap water used in this study has been assayed on a regular basis and contained an average free chlorine concentration of 0.02 mg $l^{-1}$ . It was necessary to increase this concentration so that it was between 0.1 – 0.5 mg $l^{-1}$, in accordance with the guidelines set out in the protocol (Anon., 2001). To do this it was necessary to prepare a dilution (1:100) of sodium hypochlorite solution (≈15% w/v active chlorine) in sterile de-ionised water, 10 ml of which increased the final free chlorine concentration of the 10 litre laboratory tap water sample to approximately 0.28 mg $l^{-1}$. The free and total chlorine concentration was always checked prior to beginning experimental work, in order to confirm that the free chlorine concentration was in the correct range.

To each of nine one litre Duran bottles, 900 ml of the cooled, chlorine-adjusted tap water was added. The first two of these Duran bottles acted as controls for chlorine demand: 100 ml of river water was added to the first bottle, mixed well and allowed to stand for 5 minutes before the free and total chlorine content was measured. The procedure was repeated using the second Duran bottle with 100 ml distilled water in place of 100 ml river water. If the chlorine concentration in the Duran bottle containing the river water dropped to a non-detectable level within the 5 minute period this indicated that the chlorine demand of the river water was too high, suggesting that a higher initial chlorine concentration in the tap water was required to leave a residual free chlorine level greater than 0.1 mg $l^{-1}$.

The remaining 7 Duran bottles were used for a time trial, to determine the optimum exposure time of the test sample (river water) to the chlorinated tap water. A river water volume of 100 ml was added to each of the 7 Duran bottles which were then mixed and allowed to stand for the following time intervals; 1, 1.5, 2, 2.5, 3, 3.5, and 4 minutes. To neutralise the chlorine, 1 ml of 18% w/v sodium thiosulphate solution was added at the end of the time interval. A 10 ml aliquot from each of the 7 treated samples was membrane-filtered and then cultured on MLSB (4 h 30°C, 14 h 37°C) to yield a presumptive total coliform result. All Duran bottles were stored at 4 – 8°C until required.

After incubation, all samples which achieved a target organism presumptive count of 30 – 90 CFU/10 ml were kept for further dilution to prepare replicate test samples. For each sample having counts of organisms within the target range a 1:10 dilution was prepared to provide several one litre samples each containing 10 replicate 100 ml

simulated chlorine-stressed water samples suitable for testing the novel methods and the existing standard method in parallel. This method was repeated with river water samples collected from a range of sites and with sewage samples from various treatment stages, obtained from the Northumbria Water site at Howden, Tyne and Wear.

**Procedure for direct comparison of new membrane filtration methods and UK standard method using simulated water samples**

A total of four 100 ml replicates of each simulated chlorine-stressed water sample were required for membrane filtration using standard UK procedures (Anon., 1994). These were treated as follows:

(i) The first filter was placed onto an absorbent pad soaked in 3 ml of MLSB (Anon., 1994). This was incubated for 4 hours at 30°C followed by 14 hours at 37°C.

(ii) The second filter was placed onto an absorbent pad soaked in 3 ml of MLSB. This was incubated for 4 hours at 30°C followed by 14 hours at 44°C.

(iii) The third filter was placed onto an absorbent pad soaked in 3 ml of mMLSB 1. This medium was identical to MLSB except that the two glycoside substrates, CHE-GUR (0.3 g $l^{-1}$) and X-GAL (0.08 g $l^{-1}$) replaced lactose and phenol red. Ferric ammonium citrate (0.5 g $l^{-1}$) was included for the chelation of cyclohexenoesculetin, along with the ß-galactosidase inducer IPTG (30 mg $l^{-1}$) and sodium pyruvate (0.5 g $l^{-1}$). This was incubated for 4 hours at 30°C followed by 14 hours at 37°C. Supplementation with sodium pyruvate, which degrades peroxides and superoxides, results in media which are less harsh to chlorine-stressed bacteria,

allowing for improved recoveries (Sartory, 1995). Such pyruvate supplementation has been successfully used to give higher colony counts on media for *E. coli* and coliforms (Calabrese and Bissonnette, 1990a, 1990b).

(iv) The fourth filter was placed onto an absorbent pad soaked in 3 ml of mMLSB 2. This medium was identical to MLSB except that X-α-GAL (0.08 mg $l^{-1}$) replaced lactose, and phenol red was also omitted. Sodium pyruvate (0.5 g $l^{-1}$) was included as before. This was incubated for 4 hours at 30°C followed by 14 hours at 37°C.

In later experiments mMLSB 1 and mMLSB 2 were modified to contain an increased concentration of indoxylic substrate (X-GAL and X-α-GAL) at 0.2 g $l^{-1}$, to give mMLSB 1' and mMLSB 2'.

**Reading cultures and confirmation of indicator organisms.**

**Coliforms**

As previously mentioned, coliforms are defined as oxidase-negative, facultatively anaerobic, Gram-negative rods which possess the ß-galactosidase gene. In the context of the traditional membrane filtration method they are defined as oxidase-negative, facultatively anaerobic, Gram-negative rods, which produce yellow colonies on MLSB medium at 37°C, and which are able to produce acid from lactose peptone water within 24 hours at 37°C.

(1) **MLSB at 37°C**

A total count was performed on the number of yellow colonies present on each filter membrane. These were regarded as presumptive coliforms, subject to confirmatory tests. All presumptive positive colonies or 10, whichever was the lesser, were then subcultured to Columbia agar (LabM, Bury, UK). This is a slight deviation from subculturing onto nutrient agar (NA) which is the medium recommended in the UK standard method (Anon., 1994). However, any non-selective growth medium would be suitable for this purpose, the aims of which are to obtain a pure culture and to grow organisms on a medium which does not contain any compound that is converted to acid, since this may result in a false-negative reaction of the oxidase test. After overnight incubation each subculture was then tested as follows:

a) Oxidase test: This is based on the bacterial production of a cytochrome oxidase enzyme. N,N,N,N-tetramethyl-*p*-phenylenediamine is oxidised to a coloured compound by oxidised cytochrome c, which in turn changes to reduced cytochrome c. Accordingly, the test reagent acts as an artificial electron donor and does not react directly with the enzyme (McFadden, 1976). A thin smear of the colony to be tested was placed on a filter paper strip soaked in reagent, a positive result being indicated by the development of a deep purple colour within 10 seconds and a negative result being indicated by a pale yellow colour.

b) Incubation in lactose peptone water (LPW) at 37°C for 24 hours to detect acid production (phenol red indicator).

c) Incubation in LPW at 44°C for 24 hours for detection of acid production at elevated incubation temperatures (phenol red indicator).

d) Incubation in tryptone water (TW) at 44°C for 24 hours, followed by addition of 1 drop of Kovac's reagent to examine for indole production. Tryptophan present either as an ingredient of the medium, or produced in the course of peptone breakdown, is split enzymatically into indole, pyruvic acid and ammonia. Being a volatile product, indole can be detected on addition of Kovac's reagent by a red complex formed in the upper solvent layer of the medium (McFadden, 1976).

Any oxidase-negative colonies demonstrating acid production in LPW at 37°C were regarded as confirmed coliforms. Any confirmed coliforms that demonstrated acid production and indole production at 44°C were regarded as confirmed *E. coli* (Anon., 1994).

(2) **MLSB at 44°C.**

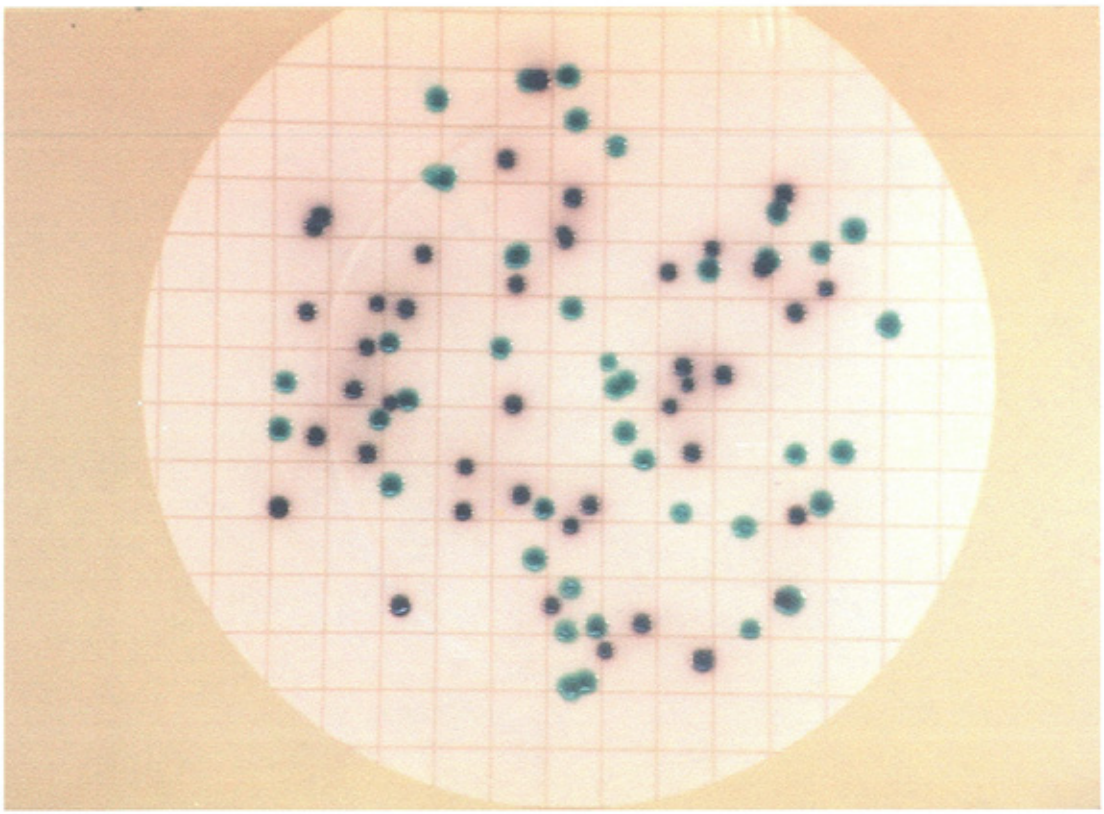
The counting and confirmatory procedure for these colonies was performed exactly as described above in part (1), above. Any oxidase-negative subcultures demonstrating acid production in LPW were regarded as thermotolerant coliforms. Any thermotolerant coliforms that demonstrated indole production at 44°C were regarded as confirmed *E. coli.*

(3) **mMLSB 1 at 37°C**

A total count was performed on all of the blue (ß-galactosidase-positive) and black (ß-glucuronidase-positive) colonies present on the filter (see Figure 3.1). These were regarded as presumptive coliforms, subject to confirmatory tests. Black colonies were

recorded as presumptive *E. coli*. Blue colonies (all or 10, whichever was least) were then subcultured and subjected to confirmatory tests exactly as described above in part (1). All, or 10 (whichever was least), of the black colonies were then subcultured and subjected to the same 4 confirmatory tests. All subcultures were accurately documented with regard to whether or not they were blue or black on the membrane filter: any blue colonies which did not confirm as coliforms (i.e. were lactose-negative in LPW) were fully identified using API 20 E (bioMérieux, Basingstoke, UK). Similarly any black colonies which did not confirm as *E. coli* were fully identified.

**Figure 3.1: Membrane filtration of a water sample on mMLSB 1 (37°C) showing the clearly distinguishable blue colonies (ß-galactosidase-positive coliforms) and black colonies (ß-glucuronidase-positive *E. coli*).**



(4) **mMLSB 2 at 37°C**

The counting and confirmatory procedure for blue colonies was performed exactly as described above in part (3). Any oxidase-negative subcultures demonstrating acid production in LPW (lactose-positive) were regarded as coliforms. Any α-GAL-positive colonies which were also oxidase-positive were identified fully using API 20 NE.

With the accurate documentation of all results it was possible to identify any major discrepancies between the four methodologies for a particular sample. For example, black colonies present on mMLSB 1 at 37°C indicated presumptive *E. coli*, and no colonies at 44°C on standard MLSB indicated no thermotolerant coliforms, i.e. no *E. coli*. In such instances it was necessary to perform full biochemical identification on the relevant colonies, e.g. the black colonies on mMLSB 1 at 37°C.

## Results and discussion

Samples 1 - 20 (Table 3.1) show results of simulated chlorine-stressed water samples originating from river water (Ouseburn - lower Tyne catchment). The counts for all water samples on all test media are in Appendix 3.2. In terms of coliform confirmation, all of the media types performed well, with confirmed coliforms always greater than 92% of the corresponding presumptive value. Throughout this section, the text refers to the number of representative confirmed coliforms or *E. coli*, i.e. the presumptive colonies (all, or 10, whichever was the lesser), removed from plates for confirmation. The figures in the Table 3.1 are for the total number of confirmed colonies based on the percentage of representative isolates confirmed to be coliforms or *E. coli*. Of the 196 representative blue colonies (ß-GAL positive) which were removed from mMLSB 1 plates as presumptive coliforms for further identification, 14 (7.1%) did not confirm to be coliforms by UK standard methods. Two of these organisms were oxidase-positive and therefore were not coliforms; for the remaining 12 organisms the negative confirmatory result was due to the fact that the organisms did not generate acid in LPW. As specified in the methods, any colony that exhibited ß-galactosidase activity but was lactose negative in LPW was identified fully by API 20 E. Of these twelve colonies: six were identified as *Enterobacter amnigenus*; four as *Enterobacter cloacae*; one as *Enterobacter asburiae*; and one as *Kluyvera* sp. All but one of these organisms (*Kluyvera* sp.) are coliforms as specified in UK definitions (Anon., 1994) and would be overlooked by UK standard methods due to their inability to ferment lactose at 37°C (lack of acid production). All of these organisms possessed ß-galactosidase as they appeared blue on mMLSB 1 and so they therefore must have

lacked the lactose permease which enables the disaccharide to enter the cell. If these organisms were included as coliforms the overall average percentage of confirmed coliforms on mMLSB 1 would be increased to 98.5%, which is the same percentage as was observed with MLSB at 37°C (Table 3.1). However, the total number of confirmed coliforms was higher with MLSB at 37°C than with mMLSB1.

The selective media for *E. coli* (MLSB at 44°C and mMLSB 1) provided encouraging results. The percentage of black colonies, confirmed as *E. coli* on mMLSB 1, was 92.2%, whereas the percentage of confirmed *E. coli* on MLSB when incubated at 44°C was only 46.3%. Furthermore, when the 51 colonies isolated as presumptive *E. coli* on mMLSB 1 (black colonies) were sub-cultured for further identification, a total of 4 were recorded as false-positives. Two of these isolates were identified by API 20 E as indole-negative *E. coli*. If these organisms were correctly included as confirmed *E. coli* then the overall percentage would increase to 96.1%. Although the percentage of confirmed *E. coli* from the presumptive positive colonies was higher for mMLSB 1 than MLSB at 44°C, in terms of actual numbers mMLSB 1 detected 20 fewer *E. coli* in total, giving only 70.1% of the count of MLSB at 44°C. Furthermore, a total of 190 presumptive coliforms isolated on MLSB at 37°C confirmed as *E. coli*; this was almost 3-times the number confirmed on MLSB at 44°C, and around 4-times greater than the number of ß-glucuronidase-positive *E. coli* detected on mMLSB 1.

The data from mMLSB 2 showed that 5 (3.2%) of the 177 representative presumptive positive coliform isolates subcultured for confirmation did not confirm as coliforms; all of these isolates were identified as *Enterobacter* spp. by API 20 E (four *E. cloacae*; one *E. sakazakii*). Again, this was due to these isolates giving a negative result when

incubated in LPW at 37°C, most likely due to the absence of lactose permease. The inclusion of these organisms as confirmed coliforms would give a confirmed recovery of 100% for coliforms on mMLSB 2, with no false-positives at the presumptive stage.

**Table 3.1: Cumulative data collected from simulated water samples 1 - 20 (river water), i.e. 20 replicates of 4 x 100 ml volumes for MF onto 4 different media.**

| Growth medium | Total number of colonies recovered | Total number of presumptive positives | Total number of confirmed coliforms | Total number of confirmed *E. coli* |
|---|---|---|---|---|
| MLSB (37°C) | 1014 | 528 | 520 (98.5%) | 190 (36.0%) |
| MLSB (44°C) | 187 | 144 | 140 (97.1%) | 67 (46.3%) |
| mMLSB 1 (ß-GAL) | 592 | 382 | 354 (92.9%) | 69 (18.0%) |
| mMLSB 1 (ß-GUR) | 592 | 51 | 51 (100.0%) | 47 (92.2%) |
| mMLSB 2 (α-GAL) | 521 | 299 | 289 (96.8%) | 93 (31.2%) |

It was noted that counts of presumptive positives on the novel media were quite low when compared with those on MLSB at 37°C, both for coliforms and *E. coli*. For example, it was apparent from the results obtained that presumptive coliform counts at this concentration were low for both X-GAL (382, or 72.3%) and X-α-GAL (299, or 56.6%) when compared with counts for yellow colonies on MLSB (528). In this study, mMLSB formulations contained indoxylic substrates (X-GAL and X-α-GAL) at a concentration of 0.08 g $l^{-1}$.Much higher concentrations of X-glycosides have been used in some earlier studies (e.g. Sartory and Howard, 1992) and so increased concentrations of 0.2 g $l^{-1}$ were used for further work, after first showing that they had no inhibitory effect in the modified media mMLSB 1' and mMLSB 2' (Table 3.2).

The data from duplicate 100 ml samples membrane filtered onto the 5 test media shows that increased concentration of X-GAL or X-α-GAL detected more presumptive positive colonies than the lower concentration that had been originally used in this investigation, in mMLSB 1 and mMLSB 2, though still not as high as the presumptive count on MLSB. In this particular study MLSB (37°C) was included only as a control medium to ensure that detection of coliforms on the novel media was at least as good as the standard medium. However, a high number of false-positive results were observed in this particular experiment when using MLSB at 37°C (Table 3.2). Overall, both mMLSB 1' and mMLSB 2' gave higher numbers of confirmed coliforms than MLSB at 37°C, representing a significant improvement over the initial formulations with lower indoxylic substrate levels (c.f. Table 3.1).

**Table 3.2: Effect of increased concentration of indoxylic substrate on detection and enumeration of coliform organisms (2 x 100 ml volumes of simulated water samples)**

| Indoxylic substrate concentration and test medium | Total number of presumptive positives | Total number of confirmed coliforms |
|---|---|---|
| X-ß-GAL (0.2 $gl^{-1}$), mMLSB 1' | 63 | 57 (90.5%) |
| X-ß-GAL (0.08 $gl^{-1}$), mMLSB 1 | 50 | 45 (90.0%) |
| X-α-GAL (0.2 $gl^{-1}$), mMLSB 2' | 54 | 54 (100.0%) |
| X-α-GAL (0.08 $gl^{-1}$), mMLSB 2 | 50 | 50 (100.0%) |
| Control: MLSB (37°C) | 73 | 51 (69.9%) |

A volume of water from the same river as that used for samples 1 - 20 was used to produce a second series of simulated chlorine-stressed river water samples for further analysis. These samples were labelled 21 - 30 and obtained from 10 replicate 4 x 100 ml volumes. Again, all media types performed well, giving high percentages for confirmed positives (Table 3.3). The increased concentration of indoxylic substrate gave improved presumptive positive counts on mMLSB 1' and mMLSB 2' when compared with MLSB at 37°C (X-GAL, 100.8%; X-α-GAL, 72.6%). Although the number of presumptive positives detected on mMLSB 2' was low, this was partly to be expected. Both MLSB and mMLSB 1' will give a positive result for *Aeromonas* spp. which are lactose fermentors (possessing ß-galactosidase). When these colonies go through the confirmatory stage they will be oxidase-positive and will not be recorded as confirmed coliforms. This is supported by the data for the novel media; of the presumptive colonies isolated on both mMLSB 1' and mMLSB 2' a slightly higher

proportion (97.9%) of mMLSB 2' colonies confirmed as coliforms than those on mMLSB 1' (95.1%). This is a possible advantage of using an α-galactosidase substrate rather than a ß-galactosidase substrate. However, it is still the case that confirmed counts were lower on mMLSB 2' than on either mMLSB 1' or standard MLSB at 37°C. As in previous samples, a high proportion of suspected false-positive coliforms were further identified as *Enterobacter* spp.. For example, of the 93 representative blue colonies subcultured for confirmation, five were regarded as false-positive results due to their inability to ferment lactose, but upon further identification four of these isolates were identified as *E. cloacae* (coliforms).

MLSB at 44°C isolated a total of 82 coliforms, 35 of which (42.7%) were confirmed as *E. coli*. In comparison, only 28 (66.7%) of the 42 black colonies isolated on mMLSB 1' were confirmed to be *E. coli*. As previously observed with samples 1 - 20 (Table 3.1) a greater number of *E. coli* (96) were isolated on MLSB at 37°C than on any other medium. This difference in confirmed counts on the novel media was slightly improved over those observed in samples 1 - 20, since 35 (at 36.5% of the MLSB count) confirmed *E. coli* were isolated on MLSB at 44°C, and 28 (at 29.2% of the MLSB count) were isolated on mMLSB 1'.

A potential problem was noted with ß-glucuronidase-positive (black colonies) on mMLSB 1' plates which included a high incidence of *Citrobacter freundii*. From the simulated samples numbered 21 - 30 in Table 3.3 analysed using mMLSB 1', providing a total of 42 representative colonies of presumptive *E. coli* (confirmed coliforms), 10 of these colonies were subsequently identified as *C. freundii* by API 20 E. In contrast, in samples 1 - 20, from a total of 51 presumptive *E. coli* only one colony was identified

as *C. freundii*. This relatively high incidence of false-positive colonies in samples 21 - 30 may be attributed to hydrogen sulphide ($H_2S$) production by certain species of *Citrobacter*. Ferric ions, present in the medium for the chelation of CHE molecules, will react with $H_2S$ to form the highly insoluble ferric sulphide which appears as a black precipitate. This reaction is the basis of Kliger's iron agar, a medium used to identify hydrogen sulphide production (Lányi, 1987) and may limit the effectiveness of CHE-based substrates. Another potential problem with regard to false positive presumptive *E. coli* results is that strong ß-galactosidase producers appear very dark blue in colour due to the intense localisation of the blue chromogen, and on a crowded filter such colonies may be misread as black, i.e. as presumptive *E. coli*. In the present study, care was taken to check that black colonies were truly ß-glucuronidase positive and not simply dark blue, due to a strong ß-galactosidase reaction. One author has observed a high incidence of ß-glucuronidase-positive *C. freundii* (Perez *et al.*, 1986). The authors evaluated a commercial rapid ß-glucuronidase test (Rosco Diagnostica, Taastrup, Denmark), which incorporates *p*-nitrophenyl-ß-D-glucuronide, a substrate which releases the yellow coloured *p*-nitrophenyl upon hydrolysis. A total of 762 clinical isolates, and 228 environmental isolates of Enterobactericeae were investigated, 75 of which were *C. freundii*. Of these 75 *C. freundii*, nine isolates confirmed as being ß-glucuronidase positive (12.0%). The authors concluded that the ß-glucuronidase test alone would be insufficient for the identification of *E. coli*.

**Table 3.3: Cumulative data collected from simulated chlorine-stressed water samples 21 - 30 (river water), i.e. 10 replicate 4 x 100 ml volumes for membrane filtration onto 4 different media**

| Growth medium | Total number of colonies recovered | Total number of presumptive positives | Total number of confirmed coliforms | Total number of confirmed *E. coli* |
|---|---|---|---|---|
| MLSB (37°C) | 326 | 266 | 266 (100.0%) | 96 (36.1%) |
| MLSB (44°C) | 90 | 82 | 82 (100.0%) | 35 (42.7%) |
| mMLSB 1' (ß-GAL) | 340 | 268 | 255 (95.1%) | 62 (23.1%) |
| mMLSB 1' (ß-GUR) | 340 | 42 | 42 (100.0%) | 28 (66.7%) |
| mMLSB 2' (α-GAL) | 270 | 193 | 189 (97.9%) | 44 (22.8%) |

Samples 31-50 represent simulated chlorine-damaged sewage water samples, using raw sewage water (samples 31 - 40; Table 3.4) and settled sewage water (samples 41 - 50; Table 3.5). The raw sewage sample was obtained from the initial stage of processing prior to screening, whereas the settled sewage sample was obtained from the end of the settling procedures prior to filtration (Figure 1.1). Table 3.4 shows comparative results for samples 31 - 40 using the novel media and MLSB. The percentage confirmation of *E. coli* on MLSB when incubated at 37°C and 44°C was

particularly poor, with a very high level of false-positive isolates. Only 17.0% and 13.9% of the total number of presumptive positive coliforms on MLSB at 37°C and 44°C respectively confirmed as *E. coli* and the majority of isolates subcultured for confirmation as *E. coli* were indole-negative and therefore confirmed as coliforms but not as *E. coli*. In contrast 100% of the black colonies sub-cultured for confirmation from mMLSB 1' were identified as *E. coli*. A possible explanation for the high incidence of false-positive colonies on MLSB at both incubation temperatures could be the presence of a mucoid organism such as *Klebsiella* sp. As the detection of presumptive-positive colonies on MLSB is reliant on a colour change of phenol red to yellow resulting from a decrease in pH, mucoid colonies can cause problems due to the diffusion of the yellow colour to surrounding colonies, which may be negative but may thereby appear positive. This problem is dramatically reduced when using a chromogenic substrate, such as X-GAL or CHE-GUR, which remains localised on the bacterial colony. As with the data for samples 1 - 30, when comparing the total numbers of *E. coli* detected, rather than the percentage confirmed from those presumed to be faecal coliforms, the numbers of colonies on MLSB at 44°C (25) and colonies on mMLSB 1' (15) confirmed to be *E. coli*, were less than those on MLSB at 37°C (42). However, 68 blue colonies isolated on mMLSB 1' were confirmed as *E. coli*. These results indicate that a substantial proportion of these *E. coli* were ß-glucuronidase-negative and therefore did not appear black on the membrane filter . In addition, 66 of the blue colonies isolated on mMLSB 2' were confirmed to be *E. coli*, which correlates well to those isolated on mMLSB 1'. Overall, the highest counts of confirmed *E. coli* for raw sewage water samples were obtained on the novel media mMLSB 1' (ß-GAL) and mMLSB 2' (α-GAL).

Of the 100 representative blue colonies subcultured from mMLSB 1' plates for confirmation as coliforms only one colony was not confirmed to be a coliform as it gave a negative result in LPW at 37°C. When identified fully using API 20 E the organism was found to be *Klebsiella pneumoniae*, suggesting this particular strain was lacking lactose permease as it was unable to ferment lactose in LPW but did possess ß-galactosidase. Furthermore, of the 100 representative blue colonies subcultured for confirmation from mMLSB 2' plates, only one colony did not confirm as a coliform, again due to a negative result in LPW at 37°C. This colony was also identified as *K. pneumoniae*.

**Table 3.4: Cumulative data collected from simulated water samples 31 - 40 (chlorine-stressed raw sewage water), i.e. 10 replicate 4 x 100 ml volumes for MF onto 4 different media types.**

| Growth medium | Total number of colonies recovered | Total number of presumptive positives | Total number of confirmed coliforms | Total number of confirmed *E. coli* |
|---|---|---|---|---|
| MLSB (37°C) | 353 | 246 | 236 (96.0%) | 42 (17.0%) |
| MLSB (44°C) | 229 | 177 | 149 (84.1%) | 25 (13.9%) |
| mMLSB 1' (ß-GAL) | 279 | 262 | 259 (99.0%) | 68 (26.0%) |
| mMLSB 1' (ß-GUR) | 279 | 15 | 15 (100.0%) | 15 (100.0%) |
| mMLSB 2' (α-GAL) | 248 | 235 | 233 (99.0%) | 66 (28.0%) |

The data for samples 41 - 50, obtained from simulated chlorine-damaged settled sewage-water samples, appeared slightly anomalous in that higher percentages of the presumptive isolates that confirmed as coliforms also confirmed as *E. coli* (Table 3.5). This occurred on all test media but was more pronounced on the novel media (mMLSB 1' and mMLSB 2') due to the fact that a fairly high number of false-positive coliform colonies (46.5%) were observed on MLSB when incubated at 37°C. Once again, the highest overall number of *E. coli* isolated was on MLSB at 37°C at 208

colonies. Only 18 colonies (8.7%) of *E. coli* were detected on MLSB when incubated at 44°C, whereas 76 *E. coli* (36.5%) were detected as black colonies on mMLSB 1'. When compared to the data for samples 21 -30, the count for MLSB at 44°C is lower while that of mMLSB 1' (ß-GUR) is slightly higher, relative to MLSB at 37°C. In contrast with the data for raw sewage water (samples 31 - 40), settled sewage water samples showed a lower count for *E. coli* in mMLSB 1' (ß-GAL) and mMLSB 2' (α-GAL), compared to MLSB at 37°C. Another interesting point about this set of data is that the membrane filters were heavily covered in non-coliform organisms. The average number of colonies recovered, both coliform and non-coliform, on all test media with previous samples (excluding samples 1 - 20 which utilised a different media formulation) was 28 CFU/plate, whereas with samples 41 - 50 the average number of colonies recovered per plate was 74, over twice the value of previous samples. Whether this can account for the large number of ß-galactosidase-positive and α-galactosidase-positive isolates confirmed to be *E. coli* on mMLSB 1' and mMLSB 2' media with samples 41 - 50 is unknown; perhaps other coliforms were inhibited by the increased presence of non-coliform organisms, or their ability to express ß-galactosidase may have been adversely affected. However, why the same inhibition would not be observed with *E. coli* is difficult to explain.

**Table 3.5: Cumulative data collected from simulated water samples 41 - 50 (chlorine-stressed settled sewage water), i.e. 10 replicate 4 x 100 ml volumes for MF onto 4 different media types**

| Growth medium | Total number of colonies recovered | Total number of presumptive positives | Total number of confirmed coliforms | Total number of confirmed *E. coli* |
|---|---|---|---|---|
| MLSB (37°C) | 808 | 398 | 213 (53.5%) | 208 (52.3%) |
| MLSB (44°C) | 106 | 21 | 21 (100.0%) | 18 (85.7%) |
| mMLSB 1' (ß-GAL) | 790 | 118 | 118 (100.0%) | 118 (100.0%) |
| mMLSB 1' (ß-GUR) | 790 | 78 | 77 (98.7%) | 76 (97.4%) |
| mMLSB 2' (α-GAL) | 1200 | 116 | 115 (99.1%) | 115 (99.1%) |

In summary, a total of 50 samples from 3 distinct sources have been counted by two reference methods (total coliforms/*E. coli* on MLSB at 37°C; faecal coliforms/*E. coli* on MLSB at 44°C) and by two novel test methods (total coliforms/faecal coliforms/*E. coli* on mMLSB 1/1'; total coliforms/*E. coli* on mMLSB 2/2'). Subsequent statistical analysis of these results has been based on the protocol to be included in the new edition of The Bacteriological Examination of Drinking Water Supplies, currently

published in draft form by the Drinking Water Inspectorate for England and Wales (Anon., 2001). This initially involved the plotting of scatter graphs for each novel test method against the relevant reference method, on linear and on logarithmic scales for count data. Figures 3.2a, 3.2b and 3.2c show scatter plots of samples 1 - 20 (counts obtained using the original low indoxylic substrate concentration), Figures 3.3a, 3.3b and 3.3c show the scatter plots of samples 21 - 50 (increased indoxylic substrate concentration). Figure 3.2a clearly shows the reference method to be better than the novel test method as 19 of the 20 data points are below the line of equivalence. This pattern is repeated in Figure 3.2b where 18 of the 20 data points are below the line of equivalence. Figure 3.2c is not influenced by the indoxylic substrate concentration as it is comparing black colonies (ß-glucuronidase positive) on mMLSB 1 with standard MLSB at 44°C. However, with this small number of samples the majority of the data points are below the equivalence line suggesting that the reference method may be slightly better than the novel test method, though there is a great deal more variation in this data set and so this interpretation is less strongly supported.

**Figure 3.2a: Scatter plot of test method 1 (blue colonies on mMLSB 1) versus reference method 1 (yellow colonies on MLSB at 37°C)**

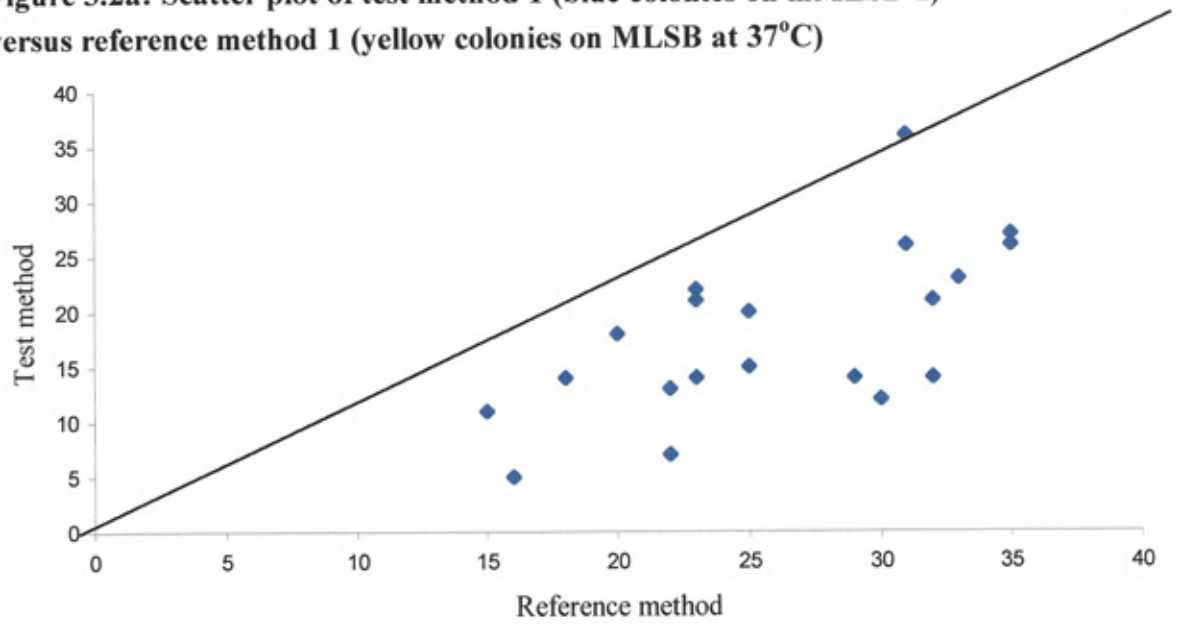



**Figure 3.2b: Scatter plot of test method 3 (blue colonies on mMLSB 2) versus reference method 1 (yellow colonies on MLSB at 37°C)**

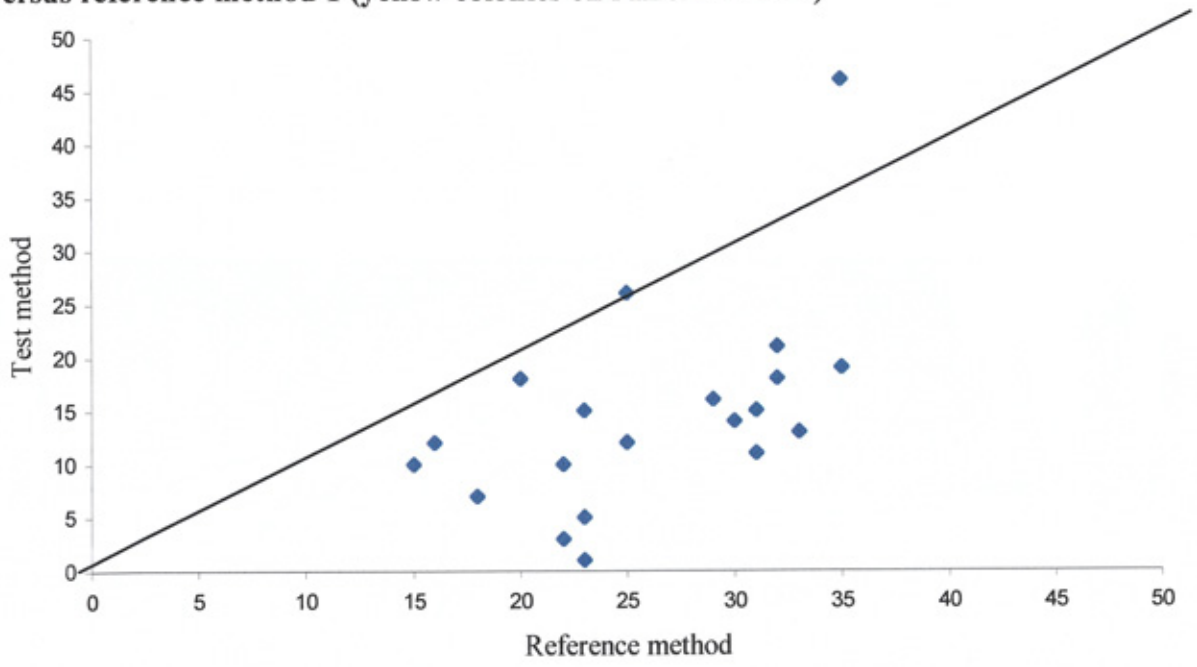


**Figure 3.2c: Scatter plot of test method 2 (black colonies on mMLSB 1) versus reference method 2 (yellow colonies on MLSB at 44°C)**

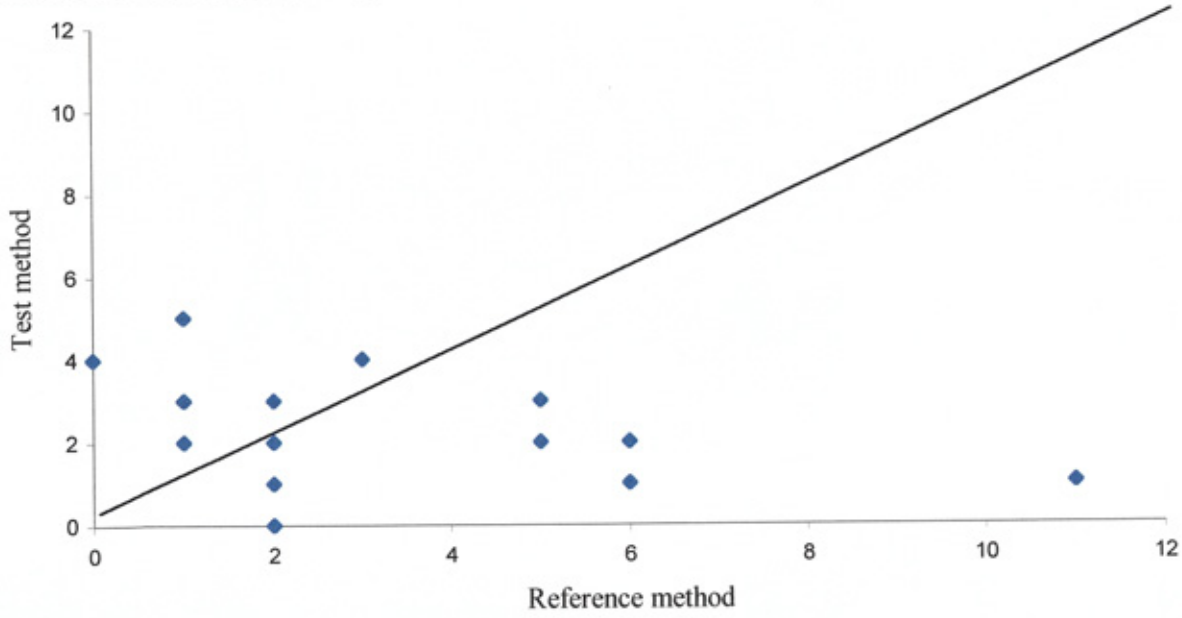



Figure 3.3a shows a marked improvement in the performance of the novel test methods with the increased indoxylic substrate concentration (c.f. Figure 3.2a). The test method appears to be broadly comparable to the reference methods in that there are as many observations above the line of equivalence as below, indicating that the test method performed as well as the reference method. Figure 3.3b shows slightly fewer data points above the line of equivalence indicating that mMLSB 2' did not perform quite as well as MLSB at 37°C. Figure 3.3c shows that these two methods performed comparably as there are a similar number of data points above and below the line of equivalence. In Figure 3.3c some differences are very large in magnitude with some counts high by one method and low by another, and vice versa. This is not unusual for microbiological samples where occasional observations display variation much greater than random (Poisson) variation, especially at low counts such as those of Figure 3.3c (Sartory, personal communication). A similar result was also obtained for samples 1 - 20 (see Figure 3.2c).

**Figure 3.3a: Scatter plot of test method 1 (blue colonies on mMLSB 1) versus reference method 1 (yellow colonies on MLSB at 37°C)**

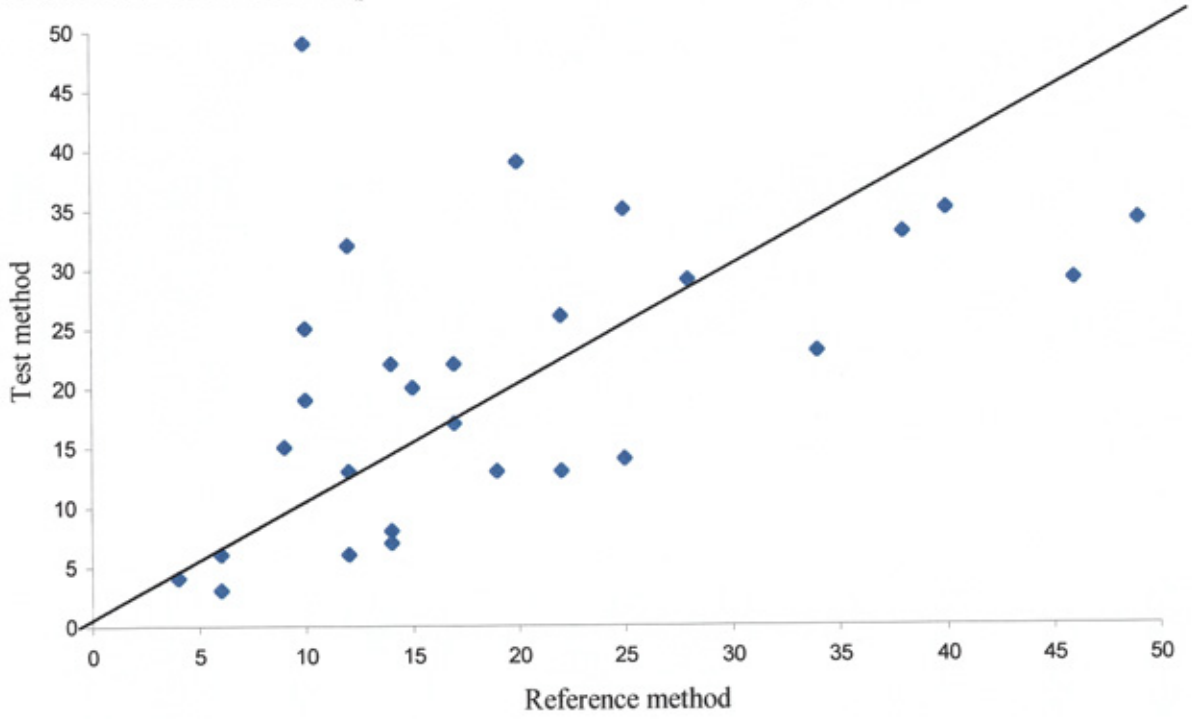



**Figure 3.3b: Scatter plot of test method 3 (blue colonies on mMLSB 2) versus reference method 1 (yellow colonies on MLSB at 37°C)**

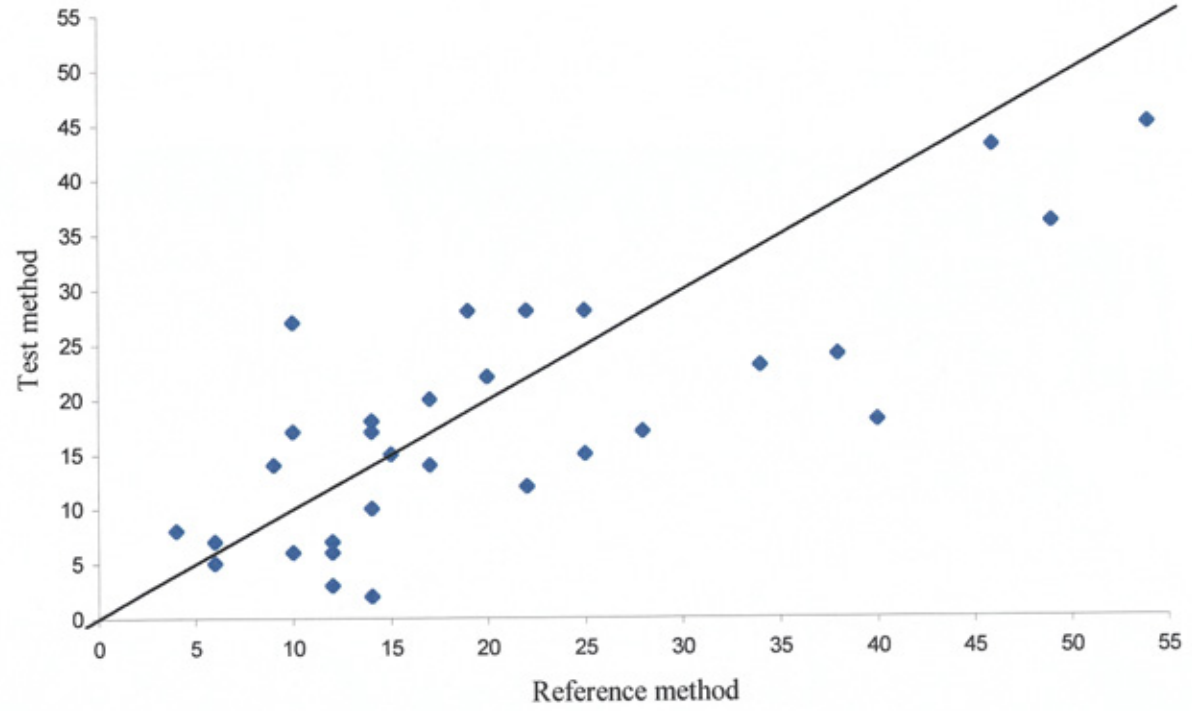
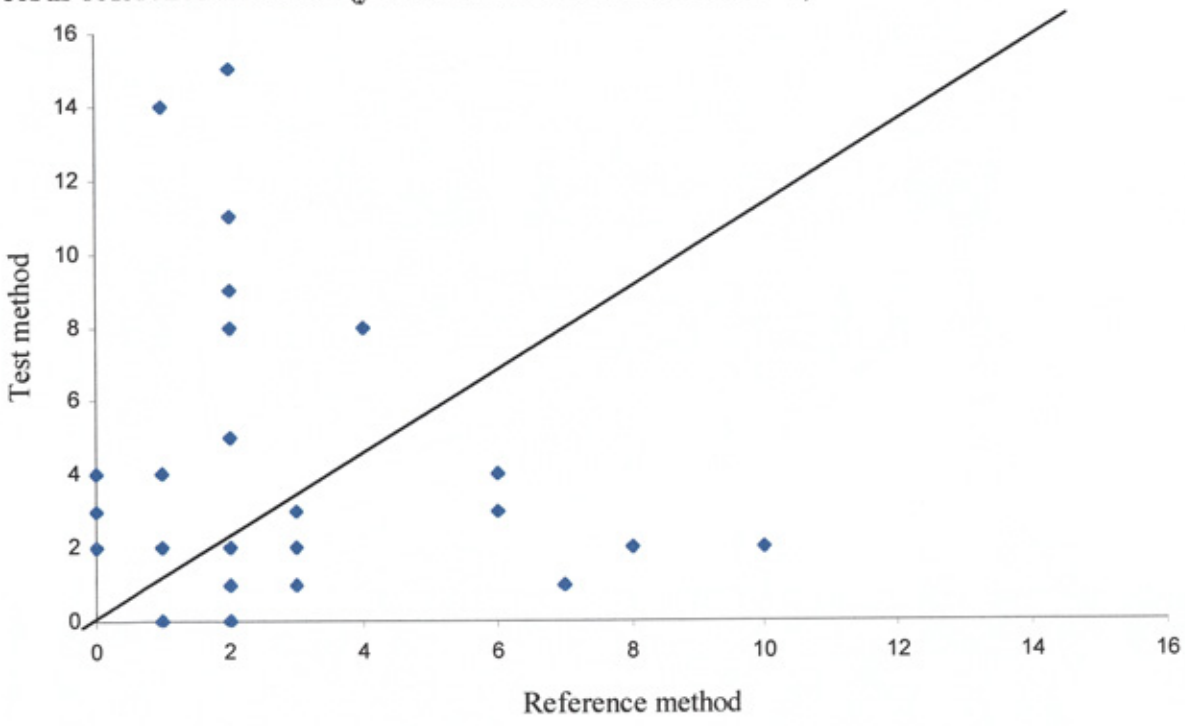


**Figure 3.3c: Scatter plot of test method 2 (black colonies on mMLSB 1) versus reference method 2 (yellow colonies on MLSB at 44°C)**




The data were then compared using non-parametric statistics, to test the null hypothesis that any discrepant paired result, where the counts are not identical, are as likely to give a higher count for the test method as for the reference method, based on the binomial distribution. This was calculated as the cumulative binomial probability (Kirkwood, 1988) using Microsoft Excel® (see Table 3.6). The data were split into sample group 1 (sample numbers 1 - 20), where the indoxylic substrate concentration (X-ß-GAL and X-α-GAL) was low, and sample groups 2-4 (samples 21 - 50, representing 3 different water samples, as described earlier), which all incorporated an increased idoxylic substrate concentration. The P values for sample group 1 have low probabilities for all comparisons of methods involving indoxylic substrates and conventional media (blue colonies on mMLSB 1 compared with yellow colonies on MLSB at 37°C; blue colonies on mMLSB 2 compared with yellow colonies on MLSB at 37°C), indicating that one method was significantly statistically different from the

other in both cases (P values of 0.00004 and 0.0004 respectively). This result indicates that the lower counts on MLSB 1 and MLSB 2 are statistically highly significant, i.e. that those two media consistently give different (lower) counts than MLSB and could not be used in place of the standard method. The comparison of MLSB at 44°C and mMLSB 1 for *E. coli* (black colonies) showed a high P value (P = 0.48), indicating that the standard UK method and the novel method did not give statistically significantly different results.

For sample groups 2, 3 and 4, when comparing test method 1 with reference method 1 (blue colonies on mMLSB 1' with yellow colonies on MLSB at 37°C), P values are high indicating no significant difference between the two methods. Where the number of replicate samples greater than the reference method is equal to the number of replicate samples less than the reference method, or when they differ by only one, P values have not been calculated as these data clearly show no significant differences between the two methods. When comparing mMLSB 2' (blue colonies) with MLSB at 37°C, all sample groups, with the exception of sample group 2 (samples 21 - 30: river water), show no significant difference between test and reference method. The data for sample 2 are difficult to explain, since the number of confirmed positive isolates with the reference method was always slightly higher than with the test method; this results in a low P value indicating that there is a significant difference between the two methods and that such a set of results are very unlikely to have arisen by random chance. Again, when comparing mMLSB 1' (black colonies) with MLSB at 44°C, one sample group (samples 41 - 50: settled sewage water) has a low P value representing a statistically significant difference between the two methods. However, in this case, the test method was better than the reference method. Overall, the analysis of the data

using non-parametric statistics, which are not as powerful as parametric statistics for detecting small differences between data sets, indicates that the test methods are not significantly different to the reference methods for most of the data sets where higher levels of indoxylic substrates were used, confirming the visual observations of the scatter graphs (Figures 3.3a-c).

**Table 3.6: P values calculated as the cumulative binomial probability for comparison of existing MLSB medium with novel chromogenic media.**

| Sample group | MLSB (37°C) v. mMLSB 1 (blue colonies) | | | | | MLSB (37°C) v. mMLSB 2 (blue colonies) | | | | | MLSB (44°C) v. mMLSB 1 (black colonies) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | > | = | < | ALL | P* | > | = | < | ALL | P* | > | = | < | ALL | P* |
| 1 | 19 | 0 | 1 | 20 | 0.00004 | 18 | 0 | 2 | 20 | 0.0004 | 11 | 2 | 7 | 20 | 0.48 |

| Sample group | MLSB (37°C) v. mMLSB 1' (blue colonies) | | | | | MLSB (37°C) v. mMLSB 2' (blue colonies) | | | | | MLSB (44°C) v. mMLSB 1' (black colonies) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | > | = | < | ALL | P* | > | = | < | ALL | P* | > | = | < | ALL | P* |
| 2 | 6 | 1 | 3 | 10 | 0.5 | 10 | 0 | 0 | 10 | 0.002 | 5 | 2 | 3 | 10 | 0.72 |
| 3 | 4 | 0 | 6 | 10 | 0.76 | 4 | 0 | 6 | 10 | 0.76 | 7 | 1 | 2 | 10 | 0.18 |
| 4 | 4 | 2 | 4 | 10 | - | 3 | 1 | 6 | 10 | 0.5 | 1 | 0 | 9 | 10 | 0.022 |
| ALL | 14 | 3 | 13 | 30 | - | 17 | 1 | 12 | 30 | 0.46 | 13 | 3 | 14 | 30 | - |

- P value not calculated when the number of replicate test samples greater than the reference method is equal to the number of replicate test samples less than the reference method, or when they differ by only one - these data clearly show no significant differences between the two methods.

Sample group 1: River Ouseburn water; Sample group 2: River Ouseburn water; Sample group 3: Raw sewage water sample; Sample group 4: Settled sewage water sample.

This combined-substrate technology with 2 distinct chromogens constitutes a particularly useful and convenient alternative method for the rapid enumeration of both *E. coli* and total coliforms within 18 hours. The method should prove to be highly cost-effective since only one filter is used for incubation at 37°C only, and the amount of substrate required is extremely small. Incorporation of an enzyme substrate into a medium allows *in-situ* detection of the enzyme, simplifying any further confirmatory testing (Sartory and Watkins, 1999). With the exception of an oxidase test, the present study has shown that confirmatory tests would be mostly unnecessary, thereby reducing labour and media costs. Furthermore, the method employed is one which is very well-known and no change to current techniques and equipment would be required.

Alonso *et al.* (1998) compared Chromocult Coliform® (CC) agar (Merck, Darmstadt, Germany) with US standard methods membrane filtration faecal coliform medium (mFC) for faecal coliform detection. The chromogenic medium incorporated Salmon-GAL, which generated a red colour when cleaved by ß-galactosidase and X-GUR, which generated a blue colour when cleaved by ß-glucuronidase. mFC medium can be used as a broth or as an agar and uses aniline blue as a pH indicator. The authors tested 40 water samples from 6 different sources. A total of 38 reference strains, made up of Enterobacteriaceae and Vibrionaceae (including *Aeromonas* spp.), were also included in the study. Samples were diluted as appropriate and membrane filtrered in duplicate onto CC agar and mFC agar. All plates were incubated at 44.5°C for 24 h. This work was repeated using a modified method involving a pre-incubation at 37°C for 2 h prior to incubating at 44°C for a further 22-24 h. The authors do not state whether replicate samples of the original method were used for this modified method.

Statistically the authors found no significant difference between faecal coliform counts obtained with the two media and the two incubation procedures. They observed that the ability of some coliforms to produce ß-galactosidase was inhibited at 44.5°C and that the growth of *Aeromonas* spp, was also inhibited at this elevated temperature (with the exception of *A. jandaei*). The authors also observed a high level of false-negative *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp. and *E. coli* when incubated at 44.5°C and suggest this is due to the physiological condition of the faecal coliform isolates resulting in non-expression of ß-galactosidase and ß-glucuronidase at the elevated temperature. This incidence of false-negative occurrences is particularly relevant as the samples included in the investigation had not been subjected to stresses such as chlorination which may lead to a much higher level of false-negative isolates.

Sartory and Howard (1992) described a solid medium, membrane lactose glucuronide agar (mLGA), for the simultaneous detection of *E. coli* and coliforms. The authors reported that the novel medium detect significantly more coliforms than the standard medium, MLSB, and that the confirmation rate for *E. coli* was substantially higher (98.6%) than for MLSB at 44°C (70.6%). This work was repeated by Walter *et al.* (1994) in an attempt to validate the findings of Sartory and Howard (1992). Walter *et al.* observed an identical confirmation rate for presumptive *E. coli* colonies of 92% for both test media (mLGA and MLSB). Furthermore, the authors reported that significantly more *E. coli* were detected using MLSB ($P < 0.05$) regardless of the bacterial load of the sample. Possible reasons for the anomalies observed in these studies are batch-to batch variation of media and chromogenic substrates, or differences in the physiological characteristics of organisms from different source waters. Martins, *et al.* (1993) suggested that *E. coli* may be phenotypically ß-

glucuronidase-negative on initial isolation, although it has the genetic capability to express the enzyme. These studies highlight the need for the testing of a large number of waters from a wide range of sources

US standard methods (Anon., 2000) recommend an elevated incubation temperature of 44.5°C to inhibit the growth of non-faecal coliforms. Frampton and Restaino (1993) indicated that a wide range of factors influence the activity of ß-glucuronidase, including incubation temperature. Alonso *et al.* (1999) suggested an incubation temperature of 41°C was most suitable for isolation of *E. coli*. Observations from this study (see Table 3.3 and 3.5) suggest that a higher number of *E. coli* can be isolated when incubated on MLSB at 37°C than on MLSB at 44°C. Why a comparatively high *E. coli* count was not obtained on mMLSB 1/mMLSB1', which were also incubated at 37°C, is unclear.

It is noteworthy that counting these intensely-coloured colonies is significantly easier than counting colonies isolated by traditional methods, this has been observed with other chromogenic media (Gaudet *et al.*, 1996; Merlino *et al.*, 1996). Brenner *et al.* (1993) observed some diffusion of the blue colour around ß-glucuronidase-positive colonies grown on filters placed on MI agar, but still observed that blue colonies were clearly visible when present with a background flora that was too numerous to count. When using MLSB, colony counting was often difficult, largely because the acid derived from lactose fermentation tends to spread and interfere with neighbouring non-fermenting colonies, and because mucoid colonies of lactose-fermenting coliforms, such as some *Klebsiella* spp., can also disguise neighbouring colonies. With both CHE-GAL and X-GAL the coloured products are completely localised within the

colony over a long period of time allowing positive colonies to be easily identified within mixed populations.

Finally, the results achieved for coliform enumeration should be in concordance with new definitions based on enzyme activity in contrast with more traditional methodologies based on lactose fermentation (Anon., 1994; Anon., 2000). In a review of the future of conventional culture for water quality assessment by Sartory and Watkins (1999), the authors suggested that any new techniques must be validated extensively before they can be accepted for routine monitoring of water and that the validation must show that the new technique gives equivalent results. They also indicate that 'seeding' water samples with sewage or river water to simulate drinking water samples is insufficient as target organisms will not have been subjected to water treatment and disinfection. However, in this study, the reduced counts on some of the novel media compared to UK standard methods (e.g. Table 3.5) give some cause for concern and warrant further study, based on a larger number of samples from a wider range of sources.

# CHAPTER FOUR

## Evaluation of fluorogenic galactoside and glucuronide substrates for the detection of coliforms and *Escherichia coli*

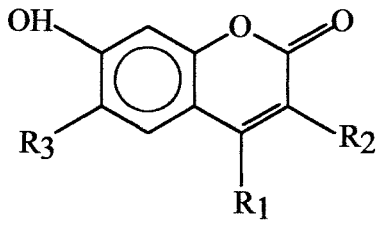
## Backgound

The hydrolytic activities of the glycosidase enzymes have been extensively studied for the differentiation of Enterobacteriaceae, facilitated by the use of chromogenic or fluorogenic substrates (Le Minor and Ben Hamida, 1962; Pérez *et al.*, 1986). The enzyme ß-galactosidase, which catalyses the breakdown of lactose into galactose and glucose and is mostly used for enumerating the coliform group, is discussed in detail in Chapter 2. The enzyme ß-glucuronidase catalyses the hydrolysis of a β-D-glucuronide derivative into an aglycone and glucuronic acid (Manafi *et al.*, 1991). The enzyme is believed to be involved in the decomposition of intercellular substances of host connective tissue (Dahlén and Linde, 1973), and the ability of *E. coli* to utilize uronic acids has been known for several years (Buehler *et al.*, 1951). As previously discussed, the majority of *E. coli* strains have been shown to possess β-glucuronidase (Killian and Bülow, 1976; Edberg and Kontnick, 1986; Kaspar *et al.*, 1987; Hansen and Yourassowsky, 1989). β-Glucuronidase activity is less common in *Shigella* spp., *Salmonella* spp., and *Yersinia* spp. (Frampton and Restaino, 1993). However, it has been suggested that as the genus *Shigella* is closely genetically related to *E. coli*, and as shigellosis is primarily a water-borne disease, the ability of methods incorporating ß-glucuronidase to detect this *Shigella* sp. is an advantage to public health protection (Berger, 1994).

The substrate 4-methylumbelliferyl-β-D-glucuronide (4-MU-GUR), which detects *E. coli* in commercial kits such as Colilert (Idexx, USA), is a derivative of the core molecule 7-hydroxycoumarin, also known as umbelliferone, see Figure 4.1. The most

common form of this core molecule is 4-methylumbelliferone (4-MU), and substrates based on 4-MU have been used extensively for the detection of enzymes in diagnostic microbiology (Trepeta and Edberg, 1984; Manafi *et al.*, 1991; Dealler, 1993; James, 1994; Bitton *et al.*, 1995). This is due to the ease of hydrolysis and intense fluorescence generated on release of 4-MU from the substrate by specific enzymes (Berg & Fiksdal, 1988; Shadix & Rice, 1991; Brenner *et al.*, 1993). The formation of the highly fluorescent 4-MU from practically non-fluorescent esters can indicate whether a particular microbial enzyme is present in a sample and enables time-dependant continuous monitoring of hydrolysis, in contrast to some chromogenic substrates (Chapter 2). Other derivatives of 7-hydroxycoumarin are shown in Table 4.1.

**Figure 4.1: Structure of 7-hydroxycoumarin (umbelliferone)**




$R_1$, $R_2$, $R_3$ = H

**Table 4.1: Examples of derivatives of 7-hydroxycoumarin at the positions labelled in Figure 4.1**

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 7-hydroxy-4-methylcoumarin (4-MU) | $CH_3$ | H | H |
| 7-hydroxycoumarin-4-acetic acid | $CH_2COOH$ | H | H |
| ethyl 7-hydroxycoumarin-3-carboxylate | H | $COOC_2H_5$ | H |
| 3-chloro-7-hydroxy-4-methylcoumarin | $CH_3$ | Cl | H |
| 6-chloro-7-hydroxy-4-methylcoumarin | $CH_3$ | H | Cl |
| methyl 7-hydroxycoumarin-3-carboxylate | H | $COOCH_3$ | H |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | $CH_3$ | $COCH_3$ | Cl |
| 7-hydroxycoumarin-3-carboxylic acid | H | COOH | H |
| 7-hydroxy-4-methylcoumarin-3-propionic acid | $CH_3$ | $C_2H_5COOH$ | H |

When the core molecule 4-MU is further derivatised with different acid or ester groups, or halogens, the molecules formed exhibit different intensities of fluorescence and different $pK_a$ values. The compound 4-MU shows a brilliant blue fluorescence when irradiated with long-wave UV light, excitation wavelength 365 nm, emission wavelength 440 nm. When derivatised at the C-7 position with a carbohydrate residue, for example glucuronic acid or galactose, the molecule becomes non-fluorescent. This means that upon hydrolysis by the appropriate enzyme (e.g. ß-glucuronidase or β-galactosidase), both the carbohydrate and the core molecule are released, the carbohydrate for metabolism by the organism while the core molecule then provides a fluorescent signal that an organism is "positive" for that particular enzyme.

An important feature of any coumarin-based substrate is that the substrate itself and the core molecule released by hydrolysis, should not be inhibitory to microbial growth. Although substrates based on 4-MU are used extensively in diagnostic microbiology, their toxicity has not been examined critically. Furthermore, any toxicity associated with such substrates will be of particular importance when used with environmental samples, since assays that include such substrates are expected to recover stressed organisms (Camper & McFeters, 1979; Calabrese & Bissonnette, 1990a), as discussed in Chapter 2.

An inherent disadvantage of 4-MU is its relatively high $pK_a$ value of 7.8 which causes only partial dissociation, of around 30%, to the highly fluorescent anion at the pH of the external growth medium, which is usually around pH 7.0 or lower, due to acid metabolites (Koller & Wolfbeis, 1985; Wolfbeis *et al.*, 1985). Hydroxylated coumarin molecules generate their maximum fluorescence in their anionic form, therefore a major advantage would be to synthesise a coumarin with a lower $pK_a$ resulting in a greater proportion of molecules in their anionic form at neutral pH. It is well-documented that derivatisation of the coumarin molecule at various positions significantly alters the deprotonation of the 7-hydroxyl group, thus providing scope for synthesising coumarins with a lower $pK_a$ (Goodwin & Kavanagh, 1950; Wolfbeis *et al.*, 1985).

A large number of novel coumarin-based core molecules have been synthesised with the potential to be used in a quantitative (MPN) assay. The present study has investigated their properties, such as fluorescence by establishing the excitation and

emission spectra for each coumarin-based core molecule. Toxicity and solubility were also investigated, to determine if any of the molecules would be suitable for conversion into glycoside substrates. A miniature multiple tube (MPN) method has been described by Hernandez *et al.* (1991), based on the substrate 4-MU-glucuronide (4-MU-GUR). Their study found that the performance of the miniaturised MPN assay was as good as the standard methods it was compared to, namely a 3-tube MPN assay and membrane filtration. This Chapter describes a modification of this miniaturised MPN method, to investigate whether improved substrate performance could be achieved with new 4-MU derivatives.

# Experimental objectives

1) To evaluate the fluorescent properties of 23 coumarin core molecules, including their excitation and emission spectra, and toxicity with a range of coliform organisms, and thereby establish which of these would be suitable for derivatisation into galactoside or glucuronide substrates.
2) To evaluate the most promising novel glycoside substrates with a range of coliform strains, in comparison with the commercially available alternatives, in order to decide which of these newly-synthesised glycoside substrates would be suitable for application in an MPN assay.

# Materials and methods

## Growth media, substrates, chemicals and equipment

MLSB was prepared from its constituents as listed in Chapter 3. Brain heart infusion (BHI) broth and Columbia agar were supplied by LabM (Bury, UK). Unless otherwise stated, all chemicals and solvents were obtained from Sigma-Aldrich Chemical Company Ltd., Poole, UK, which was also the source for 7-hydroxy-4-methylcoumarin, 7-hydroxy-4-methylcoumarin-ß-D-galactoside and 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside. In most cases the equipment used was the same as that described in Chapter 2 with the exception of sterile, flat-bottomed microtitre trays (Bibby Sterilin Limited, Aberbargoed, UK), and three microtitre plate readers summarised below:

### Anthos 2001 spectrophotometric microtitre plate reader (Labtech International Limited, Uckfield, UK)

This is a microprocessor-controlled photometer utilising nine halogen lamps which emit light beams through a diaphragm, an infra-red absorbance glass, a system of lenses and a narrow-band di-electric interference filter. The halogen lamps are selected with the same spectrum of emission to guarantee identical measurements in each of the nine channels. Eight photo-diodes detect the transmitted light and the ninth diode is used for regulation of constant light energy. Each measurement channel is calibrated prior to every measurement and the absorbance values were calculated by

microprocessor. In all cases an absorption wavelength of 690 nm was used. A total of 96 readings were accomplished in less than three seconds. Data processing was performed using a combination of Arcom for Windows® software (MR Electronics) and Microsoft Excel® 5.0 using a standard personal computer.

**Labtech Biolite F1 fluorescence microtitre plate reader**

(Labtech International Limited, Uckfield, UK)

This is a microprocessor controlled fluorimeter utilising a single optically-stabilised M32-type Tungsten-Halogen lamp with a single IP28 photomultiplier tube for detection. Up to six rotating filters are housed in the instrument each having a band-width of between 20 and 40 nm. Unless otherwise stated excitation and emission filters at 365/440 nm were used. The use of a single light source and a single detection system maximised reproducibility but resulted in a prolonged reading time of approximately 32 seconds for 96 wells. Data processing was performed using a combination of Biolite software (Astroscan Ltd) and Microsoft Excel® 5.0 using a standard personal computer.

**Labtech Biolite F2 fluorescence microtitre plate reader**

(Labtech International Limited, Uckfield, UK)

This fluorimeter/spectrophotometer can read microtitre trays of up to 384 wells. The reader is supplied with four excitation and four emission filters enabling the reader to analyse UV spectra excitation in the range 300 nm to 650 nm, and emission in the wavelength range 350 nm to 700 nm. The F2 can gather experimental data from

kinetic assays requiring a temperature-controlled environment up to 50°C, and can read absorbance and fluorescence data from the same microtitre plate at specified time intervals throughout the assay. The F2 also includes a data reduction program for Windows® so that data can be fully processed, including graphical representation, without the need to transfer the data to an Excel® spreadsheet.

**Analysis of coumarin core molecules for fluorescence properties**

A total of 23 7-hydroxycoumarin derivatives were synthesised (Table 4.2). To establish the effect of pH on the fluorescence of each coumarin molecule, 1 mol $l^{-1}$ stock solutions were prepared for each core molecule in 1 ml dimethylsulfoxide (DMSO). A one-litre stock of potassium phosphate buffer (pH 7.0) was prepared at double strength (0.2 ml $l^{-1}$) in a Duran bottle and dispensed into 9 volumes of 100 ml. The pH of each of these volumes was adjusted accordingly so that phosphate buffers were obtained over the pH range 4.0 - 8.0, increasing in pH intervals of 0.5. Solutions of each different pH were then dispensed into 25 aliquots of 3 ml. Once dissolved, 10 µl of each coumarin stock solution was added to a 3 ml aliquot of phosphate buffer at each pH in the range to be tested (3000-fold dilution of original coumarin stock solution). Triplicate 100 µl aliquots of each buffered coumarin solution were added to microtitre trays, including a buffer control at each pH and a DMSO control (3000-fold diluted) at each pH. The fluorescence was read using the Labtech Biolite F1 plate reader at an excitation wavelength of 365 nm and an emission wavelength of 440 nm.

**Table 4.2: Coumarin core molecules analysed for fluorescence and toxicity.**

| Coumarin | Relative molecular mass ($M_r$) |
|---|---|
| 7-hydroxy-4-methylcoumarin | 176.17 |
| methyl 7-hydroxycoumarin-4-acetate | 234.20 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 232.27 |
| 7-hydroxycoumarin-4-acetic acid | 220.18 |
| 6-chloro-7-hydroxy-4-methyl-3-octylcoumarin | 322.83 |
| 4-benzyl-7-hydroxycoumarin | 238.24 |
| 3-acetyl-6-hexyl-7-hydroxy-4-methylcoumarin | 302.37 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 234.20 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 210.61 |
| 3-benzyl-6-chloro-7-hydroxy-4-methylcoumarin | 286.71 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 282.68 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 251.68 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 210.61 |
| methyl 7-hydroxycoumarin-3-carboxylate | 220.18 |
| 6-hexyl-7-hydroxy-4-methylcoumarin | 260.33 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 252.65 |
| ethyl 4,8-dimethyl-6-ethyl-7-hydroxycoumarin-3-propanoate | 318.36 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 204.22 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 266.72 |
| 7-hydroxycoumarin-3-carboxylic acid | 206.15 |
| 7-hydroxy-3-methoxyacetylcoumarin | 234.20 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 218.20 |
| 7-hydroxy-4-methylcoumarin-3-propionic acid | 247.23 |

**Analysis of coumarin core molecules for toxicity**

To establish any inhibitory effects on the growth of coliform bacteria, each coumarin stock solution (1 mol $l^{-1}$) was diluted in brain heart infusion (BHI) broth, to achieve the following concentrations; 1.0, 0.25 and 0.0625 mmol $l^{-1}$. The pH of each of these coumarin broths was checked prior to filter-sterilisation and adjusted to pH 7.4 +/- 0.2 if necessary. All subsequent dilutions were carried out aseptically using sterile BHI broth. Twelve coliform organisms were included in the toxicity assay, with individual type strains of the three coliforms: *Escherichia coli* (National Collection of Type Cultures 10418); *Klebsiella pneumoniae* (NCTC 10896); *Citrobacter freundii* (NCTC 9750). Three wild strains of each of the three species, isolated from patients at Freeman Hospital, Newcastle upon Tyne (FRHECO1, FRHECO2, FRHECO3, FRHKPN1, FRHKPN2, FRHKPN3, FRHCFR1, FRHCFR2, FRHCFR3) were also included. The strains were cultivated on Columbia agar at 37°C for 18 h; each strain was then harvested and suspended in BHI broth to a density equivalent to a McFarland Standard of 1.0 ($\approx 3 \times 10^8$ CFU/ml). Each suspension was then diluted in sterile BHI broth (1:1000) to a density of $\approx 3 \times 10^5$ CFU/ml. Plate counts were performed on Columbia agar to confirm bacterial numbers.

A volume of 50 µl of diluted bacterial suspension was added to an equal volume of coumarin broth, in triplicate in microtitre wells, resulting in final concentrations of coumarin and numbers of organism of 0.5, 0.125, 0.03125 mmol $l^{-1}$ and $\approx 1.5 \times 10^5$ CFU/ml respectively. Appropriate coumarin-free and bacteria-free controls were included in each microtitre tray, which also included DMSO solvent controls prepared at the same concentrations as the coumarin stock solutions. Growth was monitored by

measuring absorbance at 690 nm at 30 min intervals for the initial 6 h of incubation at 37°C, with shaking. Microtitre trays were then incubated for a further 18 h at 37°C without shaking, to provide a final absorbance reading at 24 h.

A more extensive investigation of potential growth inhibition was carried out with 4-MU, incorporating a broader range of concentrations. Stock 4-MU (1 mol $l^{-1}$) was diluted in BHI broth to achieve the following concentrations; 2.0, 1.0, 0.5, 0.25, 0.125, 0.064, 0.032, 0.016, 0.008 and 0.004 mmol $l^{-1}$. Eight strains of *E. coli* were used in this experiment, seven wild strains (FRHECO1-7) and one type strain (NCTC 10418). All strains were cultivated and harvested as previously described. Strains were diluted to the same bacterial density as the previous experiment ($\approx 3 \times 10^5$ CFU/ml), then aliquots of 50µl were added to an equal volume of each coumarin dilution in triplicate in microtitre format, resulting in the final concentrations of coumarin and organism to be half of those listed above. Controls were included as described previously.

**Analysis of the toxicity of coumarinic galactosides and ß-galactosidase activity with various coliform bacteria.**

Four of the most promising coumarin molecules were subsequently derivatised to form galactosides (Table 4.3). All galactoside substrates were used at an equivalent concentration of 1 mmol $l^{-1}$ to assess their fluorescence and any potential inhibitory effect on bacterial growth.

**Table 4.3: ß-galactosidase fluorogenic substrates based on 4-MU derivatives synthesised for comparative analysis with 4-MU-GAL.**

| ß-galactosidase substrate | Relative molecular mass ($M_r$) |
|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-β-D-galactoside | 396.20 |
| 6-chloro-7-hydroxy-4-methylcoumarin-β-D-galactoside | 372.81 |
| 7-hydroxycoumarin-3-caboxylic acid-β-D-galactoside | 368.35 |
| 7-hydroxy-4-methylcoumarin-3-propionic acid-β-D-galactoside | 409.43 |
| 7-hydroxycoumarin-4-acetic acid-β-D-galactoside * | 382.30 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside (4-MU-GAL)* | 338.37 |

* Obtained commercially (Sigma-Aldrich Chemical Company Ltd., Poole, UK).

Modified membrane lauryl sulphate broth (mMLSB) was used for the fluorescence assay and for establishing whether the galactosides exhibited any inhibitory effect on bacterial growth. mMLSB was prepared in modified format as previously described (Chapter 3), to contain no phenol red and no lactose. An amount equivalent to 0.01 mmol of each substrate was weighed out and dissolved in 10 ml of mMLSB, with heating, if necessary, to give a concentration of 1 mmol $l^{-1}$. Once dissolved, the ß-galactosidase inducer isopropyl-β-D-thiogalactoside (IPTG) was added (Diehl, 1991) at 60 mg $l^{-1}$ to each substrate broth, the pH of each substrate/IPTG broth was checked and altered to 7.4 +/- 0.2, if necessary, followed by filter-sterilisation.

As for the toxicity studies of the core molecules, the same 12 coliform organisms were harvested from Columbia agar after 18 h incubation at 37°C. Each strain was suspended in mMLSB to a density equivalent to a McFarland Standard of 1.0 (≈ 3 x $10^8$ CFU $ml^{-1}$) and then diluted (1:50) in mMLSB to a suspension density of approximately 6 x $10^6$ CFU $ml^{-1}$. Plate counts on Columbia agar were taken to confirm bacterial numbers. Each substrate/IPTG broth was added to microtitre trays in 50 µl aliquots, followed by the addition of an equal volume of diluted bacterial suspension, resulting in final concentrations of 0.5 mmol $l^{-1}$ substrate, 30 mg $l^{-1}$ IPTG and 3 x $10^6$ CFU $ml^{-1}$ bacterial density. Appropriate substrate-free and bacteria-free controls were included in each microtitre tray. All experimental work was carried out in triplicate. Trays were incubated at 37°C with shaking, monitoring both absorbance (690 nm) and fluorescence (365/440 nm) hourly for 6 h, for growth and substrate hydrolysis respectively. Trays were then incubated for a further 18 h at 37°C without shaking, to record a final (24 h) reading of both fluorescence and absorbance.

**Evaluation of fluorogenic glucuronide substrates**

Four of the coumarin molecules were subsequently derivatised to form glucuronide substrates (Table 4.4). As before, the glucuronide substrates were all used at an equivalent concentration of 1 mmol $l^{-1}$ (double strength) to assess their fluorescence and any potential inhibitory effect on bacterial growth.

**Table 4.4: ß-glucuronidase fluorogenic substrates based on 4-MU derivatives synthesised for comparative analysis with 4-MU-GUR**

| ß-glucuronidase substrate | Relative molecular mass ($M_r$) |
|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-glucuronide | 410.30 |
| 6-chloro-7-hydroxy-4-methylcoumarin-β-D-glucuronide | 386.71 |
| methyl 7-hydroxycoumarin-3-caboxylate-β-D-glucuronide | 396.28 |
| benzyl 7-hydroxycoumarin-3-carboxylate-ß-D-glucuronide | 472.28 |
| 7-hydroxy-4-methylcoumarin-ß-D-glucuronide (4-MU-GUR)* | 352.27 |

*Obtained commercially (Sigma-Aldrich Chemical Company Ltd., Poole, UK).

As in previous toxicity and fluorescence studies, mMSLB was used. This study was carried out in exactly the same way as previously described for the galactoside substrates except that the organisms consisted of six *E. coli* strains, five of which were wild strains (FRHECO2, 4, 5, 6 and 8) and one type strain (*E. coli* NCTC 10418). IPTG was not used as no equivalent gratuitous inducer of ß-glucuronidase is available for inclusion in the test assay.

# Results and discussion

## Analysis of coumarin core molecules for fluorescence properties and toxicity.

Figure 4.2 shows the fluorescence data of the coumarin molecules derivatised with ester groups, compared to 4-MU. Appendix 4.1 includes the fluorescence data for all coumarin core molecules at each pH. Fluorescence was plotted against pH for each coumarin. An extensive investigation of data at each pH was carried out noting any coumarins which gave erratic data, indicating problems with the solubility of the coumarin derivative (see Appendix 4.1 for fluorescence data of all coumarin core molecules at each pH). Fluorescence data show that some coumarin core molecules were substantially more fluorescent than 4-MU at pH 7.0. For example, at pH 7.0 the fluorescence of ethyl 7-hydroxycoumarin-3-carboxylate was 2.5-times greater than the fluorescence exhibited by 4-MU. This difference was even more pronounced at pH 6.0, the fluorescence of ethyl 7-hydroxycoumarin-3-carboxylate being more than 7.5-times greater than that exhibited by 4-MU. Furthermore, both ethyl 7-hydroxycoumarin-3-carboxylate and methyl 7-hydroxycoumarin-3-carboxylate appeared to be far more stable over a broader pH range than 4-MU. The fluorescence exhibited by these two coumarin core molecules remained above 47000 fluorescence units at pH values as low as pH 4.0. Conversely, the fluorescence of 4-MU decreased steadily as pH decreased until pH 6.0 where it remained fairly constant at around 7000 fluorescence units. However, when derivatised at the C-8 position with a methyl group, as in ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate and ethyl 4,8-dimethyl-6-ethyl-7-hydroxycoumarin-3-propanoate (Figure 4.2), the molecules were almost non-

fluorescent and therefore of no further interest for the synthesis of a diagnostic substrate.

**Figure 4.2: Effect of pH on the fluorescence of various ester derivative coumarin molecules at a concentration of 0.33 mmol $l^{-1}$, including 4-MU for comparison.**

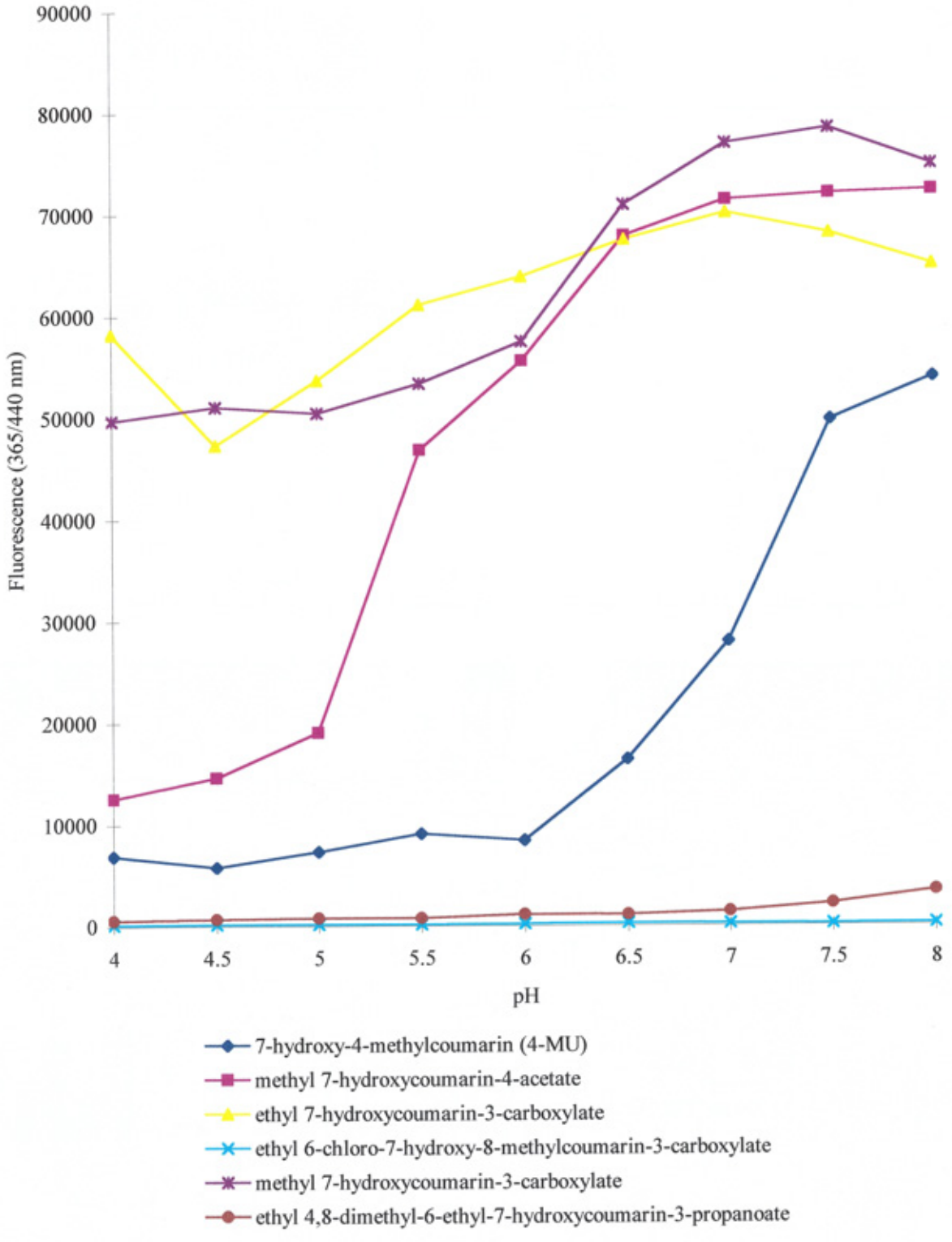


Figure 4.3 shows the fluorescence data of the coumarin molecules derivatised with acid groups in comparison with 4-MU. Two of these molecules behaved very similarly to 4-MU over the full pH range: namely 7-hydroxy-4-methyl-3-propionic acid and 7-hydroxycoumarin-4-acetic acid. However, 7-hydroxycoumarin-3-carboxylic acid was more fluorescent that 4-MU over the entire pH range included in this study. At pH 6.0 this core molecule exhibited fluorescence more than 5-times greater than 4-MU.

**Figure 4.3: Effect of pH on the fluorescence of various acid derivative coumarin molecules at a concentration of 0.33 mmol $l^{-1}$, including 4-MU for comparison.**

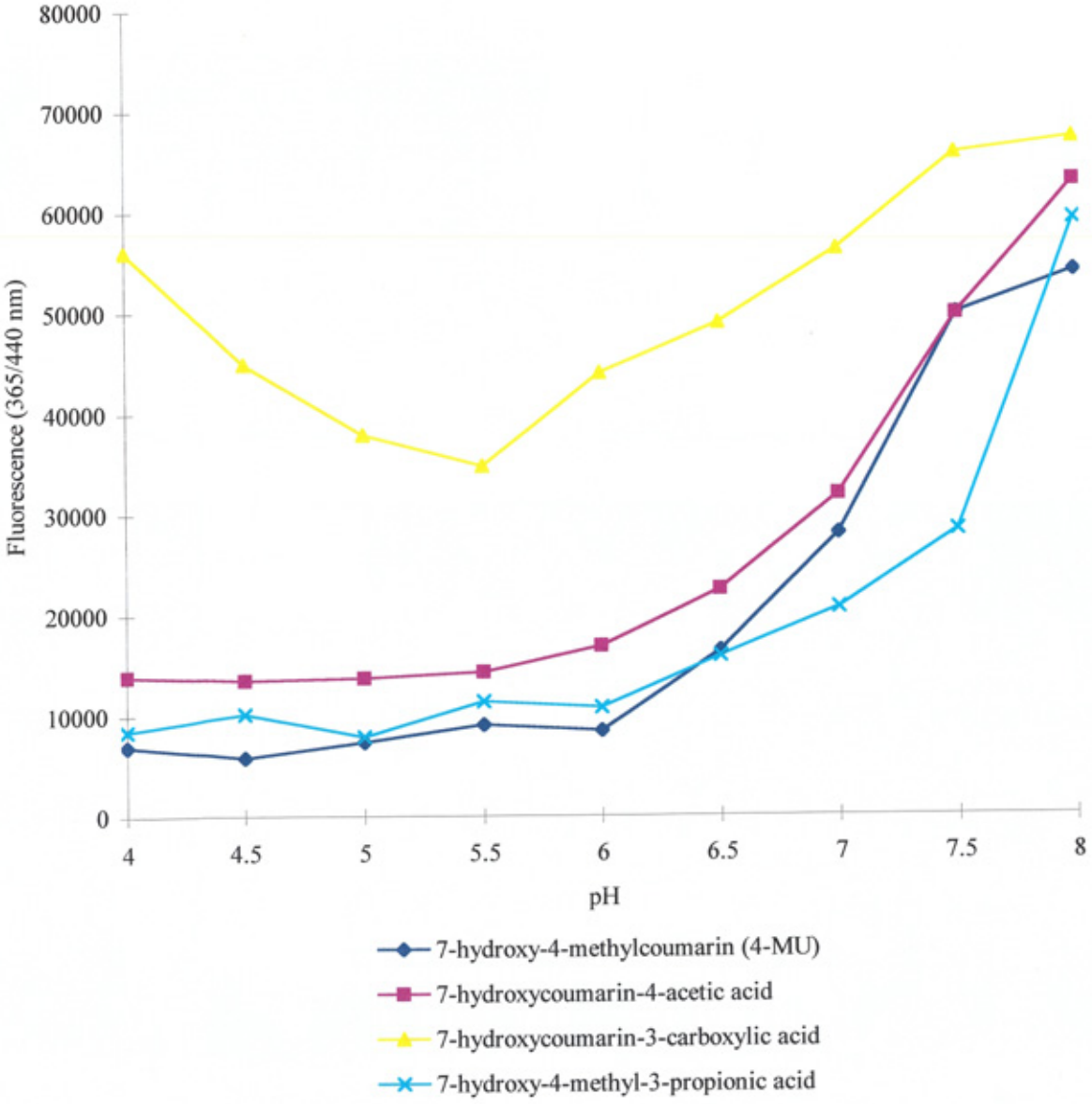


Figure 4.4 shows the fluorescence data for all of the remaining coumarin core molecules. This figure demonstrates that 5 of the novel coumarin core molecules were less fluorescent than 4-MU between pH 6.0 - 7.0, these include: 3-butyl-7-hydroxy-4-methylcoumarin; 3-chloro-7-hydroxy-4-methylcoumarin; 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin; 6-ethyl-7-hydroxy-4-methylcoumarin; and 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin. This information indicates that these coumarin molecules would not be suited for derivitisation into ß-glycoside substrates due to the fact that the fluorescence generated upon enzymatic hydrolysis of the substrate would be poorer than the commercially-available 4-MU. Three of the coumarins shown in Figure 4.4 exhibited more fluorescence than 4-MU at pH 6.5. One of these core molecules, 7-hydroxy-3-methoxyacetylcoumarin, exhibited a very stable fluorescence throughout the pH range. The fluorescence was minimally improved on that of 4-MU at pH 6.5, however, at pH 7.0 the fluorescence of this core molecule was less than 4-MU, indicating that this molecule would not offer any advantages over 4-MU for this particular application. The two remaining core molecules represented on Figure 4.4, 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin and 6-chloro-7-hydroxy-4-methylcoumarin behaved very similarly to 4-MU up to pH 6.0. At pH values higher than this the fluorescence exhibited by these two molecules was slightly increased to that of 4-MU, with approximately 1.6-time greater fluorescence at pH 7.0.

**Figure 4.4: Effect of pH on the fluorescence of various coumarin molecule derivitives at a concentration of 0.33 mmol $l^{-1}$, including 4-MU for comparison.**

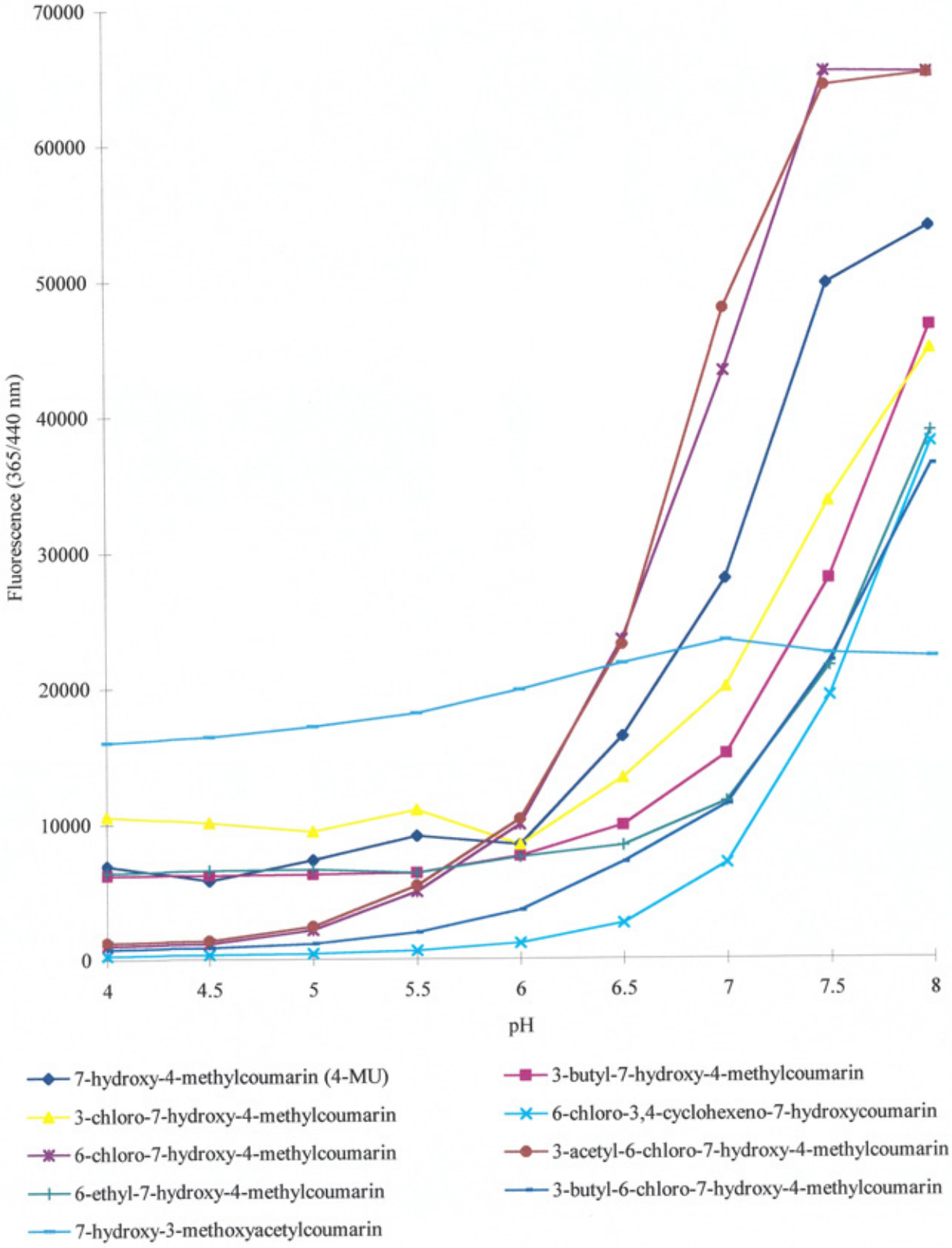


Figure 4.5 is a composite representation of all of the coumarin core molecules which showed a greater fluorescence than 4-MU between pH 6.5 and 7.5, clearly showing the potential of these molecules as fluorescent markers. Some show fluorescence greater than 6-fold higher (e.g. methyl 7-hydroxycoumarin-3-carboxylate and ethyl 7-hydroxycoumarin-3-carboxylate) than the commercially-available fluorogen at pH 6.0. This implies that less of the substrate might be used to generate the same level of fluorescence, or, that using the same concentration may allow the fluorescence generated to be detected more rapidly.

**Figure 4.5: Effect of pH on the fluorescence of various coumarin molecule derivatives which exhibited a higher fluorescence than 4-MU at pH 6.5 (all coumarins tested at a concentration of 0.5 mmol $l^{-1}$).**

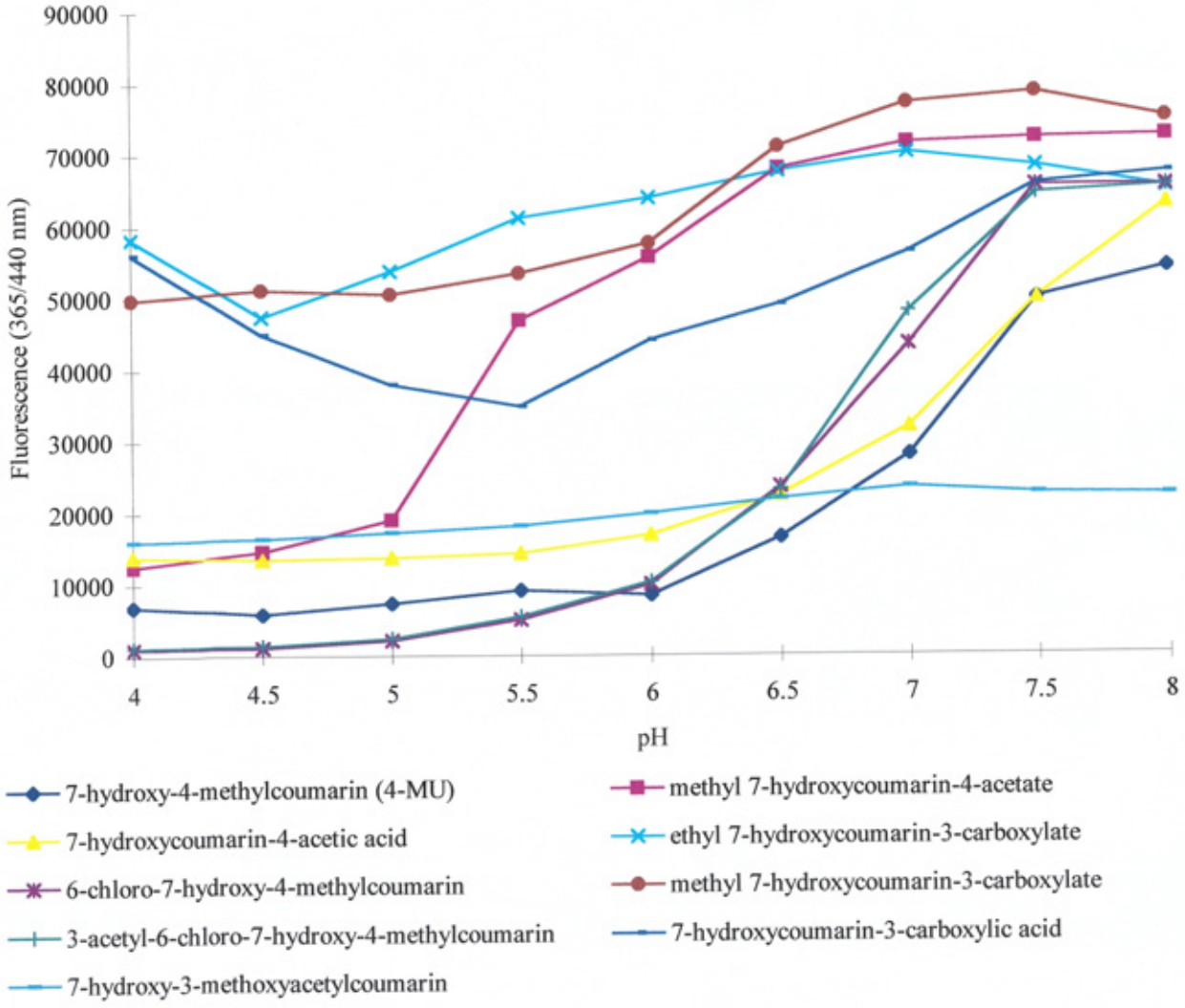


Table 4.5 shows the effect of coumarin core molecules at a concentration of 0.5 mmol $l^{-1}$ on the relative growth of a range of 12 coliform organisms: data are based on percentages of the absorbance increase of the growth control after 300 min incubation at 37°C (see Appendix 4.2). Some strains appear to be more affected by the presence of coumarin core molecules than others. For example, FRHCFR1 exhibited 100% growth when in the presence of almost all core molecules, the only exception being 3-chloro-7-hydroxy-4-methylcoumarin, where the increase in absorbance in the presence of this molecule was 68% of the growth control. The NCTC strains of *E. coli* and *K. pneumoniae* (NCTC 10418 and NCTC 10896 respectively) were more inhibited by the presence of coumarin molecules than the wild strains, with several coumarins inhibiting the growth of these strains completely. The core molecule 6-ethyl-7-hydroxy-4-methylcoumarin appeared to be the most inhibitory coumarin molecule, inhibiting growth completely in five of the twelve strains tested.

**Table 4.5: The effect of each coumarin core molecule (0.5 mmol $l^{-1}$) on relative growth (% control) of the twelve coliform organisms included in this study - based on absorbance increase at 690 nm after 300 min incubation at 37°C.**

| Coumarin core molecule | *E. coli* | | | | *K. pneumoniae* | | | | *C. freundii* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NCTC 10418 | FRH ECO1 | FRH ECO2 | FRH ECO3 | NCTC 10896 | FRH KPN1 | FRH KPN2 | FRH KPN3 | NCTC 9750 | FRH CFR1 | FRH CFR2 | FRH CFR 3 |
| 7-hydroxy-4-methylcoumarin (4-MU) | 59 | 45 | 38 | 34 | 25 | 55 | 74 | 35 | 42 | 100 | 62 | 86 |
| methyl 7-hydroxycoumarin-4-acetate | 31 | 58 | 74 | 70 | 38 | 80 | 100 | 64 | 65 | 100 | 100 | 92 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 30 | 59 | 70 | 0 | 70 | 92 | 68 | 73 | 100 | 71 | 40 |
| 7-hydroxycoumarin-4-acetic acid | 97 | 70 | 78 | 72 | 63 | 93 | 100 | 81 | 77 | 100 | 100 | 97 |
| 4-benzyl-7-hydroxycoumarin | 97 | 34 | 72 | 49 | 19 | 82 | 97 | 84 | 46 | 100 | 100 | 60 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 62 | 64 | 91 | 51 | 38 | 82 | 81 | 80 | 54 | 100 | 93 | 56 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 3 | 40 | 56 | 45 | 0 | 62 | 74 | 51 | 12 | 68 | 28 | 24 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 7 | 30 | 55 | 26 | 0 | 50 | 73 | 52 | 123 | 100 | 63 | 43 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 59 | 68 | 100 | 84 | 75 | 84 | 92 | 100 | 88 | 100 | 81 | 92 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 7 | 49 | 78 | 58 | 44 | 82 | 93 | 65 | 58 | 100 | 66 | 93 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 6 | 66 | 53 | 31 | 79 | 51 | 6 | 42 | 100 | 50 | 79 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 4 | 71 | 57 | 0 | 83 | 83 | 34 | 50 | 100 | 82 | 91 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 8 | 0 | 0 | 0 | 83 | 65 | 31 | 100 | 99 | 73 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 90 | 81 | 100 | 100 | 100 | 100 | 100 | 88 | 92 | 100 | 100 | 99 |
| 7-hydroxycoumarin-3-carboxylic acid | 83 | 91 | 100 | 100 | 94 | 100 | 100 | 94 | 65 | 100 | 100 | 100 |
| 7-hydroxy-3-methoxyacetylcoumarin | 69 | 100 | 93 | 100 | 44 | 98 | 98 | 91 | 50 | 100 | 96 | 91 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 92 | 95 | 100 | 0 | 93 | 89 | 80 | 54 | 100 | 87 | 86 |
| 7-hydroxy-4-methylcoumarin-3-propionic acid | 97 | 100 | 92 | 89 | 94 | 96 | 100 | 91 | 62 | 100 | 97 | 100 |
| Control (growth medium and solvent) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Table 4.6 shows the average increase in absorbance at 690 nm of all 12 organisms, together with the fluorescence values from each of the core molecules at pH 7.0. The data clearly show that some coumarin core molecules were substantially less inhibitory to bacterial growth than 4-MU, which gave an absorbance change just over half of the control value; for example, 7-hydroxycoumarin-3-carboxylic acid, 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin and 7-hydroxy-4-methylcoumarin-3-propionic acid all generated average increases in absorbance within 10% of the control. A total of twelve coumarin core molecules exhibited less of an inhibitory effect on coliform growth than 4-MU. These data confirmed that several substituted coumarin molecules had been synthesised which were both substantially more fluorescent than 4-MU and substantially less inhibitory to the growth of coliform organisms, and these were: methyl 7-hydroxycoumarin-4-acetate; 7-hydroxycoumarin-4-acetic acid; ethyl 7-hydroxycoumarin-3-carboxylate; 6-chloro-7-hydroxy-4-methylcoumarin; 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin; 7-hydroxycoumarin-3-carboxylic acid. The core molecule ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate was the poorest-performing coumarin, based on fluorescence and toxicity. A number of coumarins either exhibited high fluorescence but were inhibitory to bacterial growth, such as 3-chloro-7-hydroxy-4-methylcoumarin, or low fluorescence and little inhibition to bacterial growth, such as, 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin.

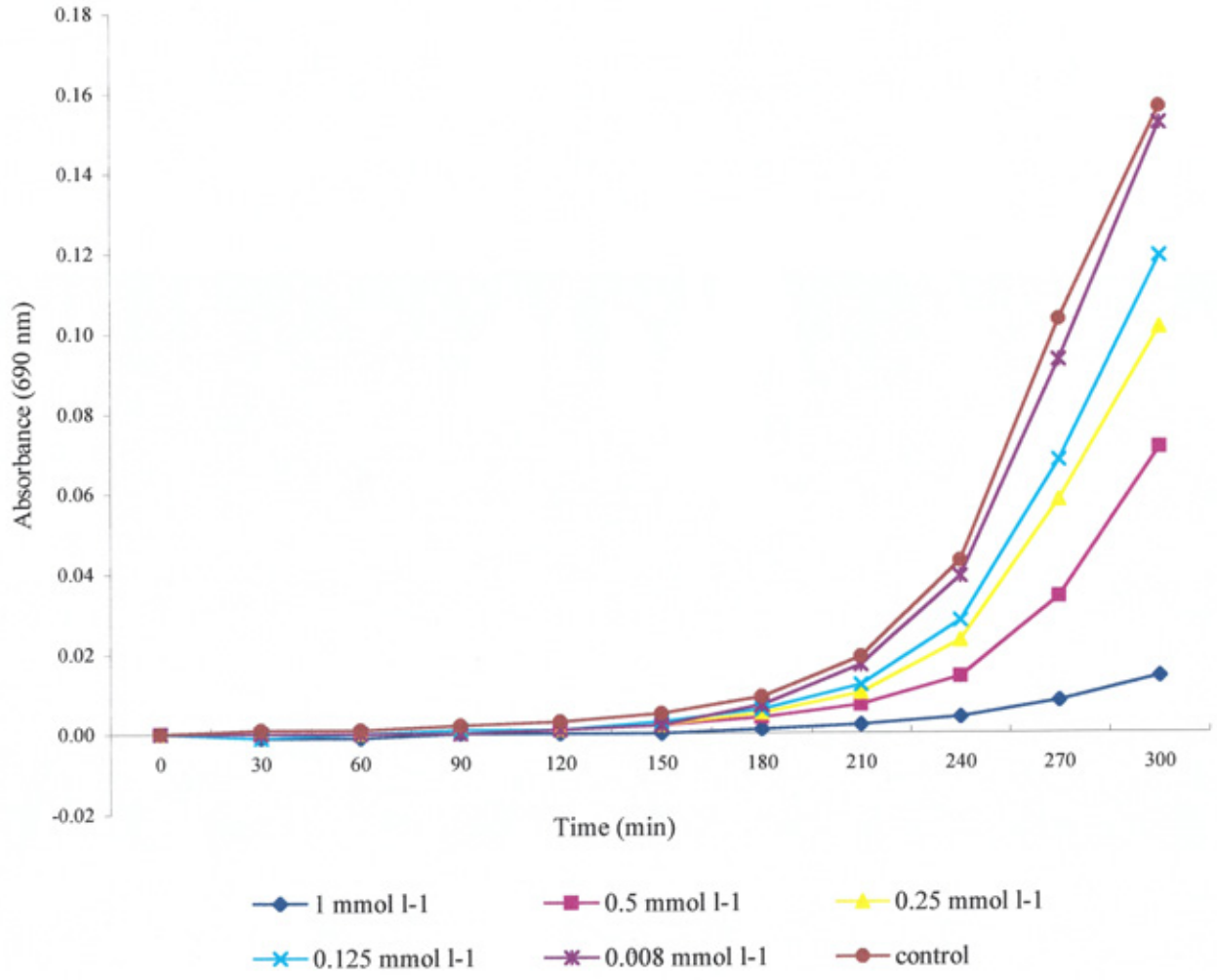
**Table 4.6: Fluorescence of 0.5 mmol $l^{-1}$ of coumarin core molecules at pH 7.0 and the effect of each core molecule on coliform growth (average of values taken from data in Table 4.5)**

| Coumarin core molecule | Fluorescence (pH 7.0 relative units) | Relative growth* (% control) |
|---|---|---|
| 7-hydroxy-4-methylcoumarin (4-MU) | 27943 | 55 |
| methyl 7-hydroxycoumarin-4-acetate | 71328 | 73 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 15052 | 56 |
| 7-hydroxycoumarin-4-acetic acid | 31823 | 86 |
| 4-benzyl-7-hydroxycoumarin | 195 | 70 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 70043 | 71 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 20021 | 39 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 189 | 52 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 7056 | 86 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 43287 | 66 |
| methyl 7-hydroxycoumarin-3-carboxylate | 76883 | 47 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 47880 | 55 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 11576 | 38 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 11356 | 96 |
| 7-hydroxycoumarin-3-carboxylic acid | 56175 | 94 |
| 7-hydroxy-3-methoxyacetylcoumarin | 23424 | 86 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 186 | 73 |
| 7-hydroxy-4-methylcoumarin-3-propionic acid | 20564 | 93 |
| Control (growth medium and solvent) | - | 100 |

*based on absorbance increase at 690 nm after 300 min incubation

A study of growth inhibition by 4-MU was carried out over a wide concentration range (see Appendix 4.3). This study showed that, with increasing concentration, 4-MU was inhibitory to the optimal growth of all 8 tested strains of *E. coli*. Figure 4.6 shows a representative set of data for growth of *E. coli* NCTC 10418 at various concentrations of 4-MU, up to a maximum of 1 mmol $l^{-1}$. At concentrations below 0.008 mmol $l^{-1}$ 4-MU exhibited minimal effect (data not shown in Figure 4.6 since the absorbance changes were equivalent to those of the control). However, growth was inhibited above this level; for example at 0.5 mmol $l^{-1}$, the increase in absorbance at 300 min was only 46% of that produced by the growth control and at 1.0 mmol $l^{-1}$ it was only 9% of the control.

**Figure 4.6: Growth of *Escherichia coli* (NCTC 10418) in the presence of various concentrations of 7-hydroxy-4-methylcoumarin (4-MU) in BHI broth**



**Analysis of the toxicity of coumarinic galactosides and ß-galactosidase activity for various coliform bacteria.**

Four of the novel coumarin molecules were subsequently derivatised to form galactosides (see Table 4.3). Both 4-MU-GAL and 7-hydroxycoumarin-4-acetic acid-β -D-galactoside were obtained commercially, therefore a total of six ß-galactoside substrates were compared at equivalent concentrations (0.5 mmol $l^{-1}$) to assess their fluorescence and any potential inhibitory effect on bacterial growth.

Table 4.7 shows the results of the studies carried out with the six ß-galactosidase substrates, including the 4 newly synthesised coumarinic galactosides, none of which exhibited an inhibitory effect on bacterial growth, based on absorbance readings at 690 nm after 6 h or after overnight incubation (see Appendix 4.4). Although 4-MU-GAL performed reasonably well, with the average increase in absorbance in the presence of the substrate being 90% and 95% that of the growth control at 6 h and 18 h respectively, these data suggest that this substrate was somewhat inhibitory to bacterial growth when compared to the novel substrates, in agreement with the data for the core molecule 4-MU (Figure 4.6). The lowest increase in absorbance, observed at both incubation times, resulted from the growth medium containing 4-MU-GAL, whereas after 18 h incubation, 3 of the 4 media containing novel substrates showed identical increases in absorbance to that of the growth control, and all were better than 4-MU-GAL at 6 h and at 18 h.. The second commercially available substrate included in this study, 7-hydroxycoumarin-4-acetic acid-β-D-galactoside, exhibited slight inhibition after 6 h incubation but showed identical increases in absorbance to the growth control after 18 h incubation.

Certain galactoside substrates generated substantially more fluorescence upon hydrolysis by the growing bacterial culture than other substrates (data taken from Appendix 4.5). Thus, the novel substrate ethyl-7-hydroxycoumarin-3-carboxylate-galactoside (EHC-GAL), generated the maximum fluorescence of the coumarinic galactosides after 6 h incubation (Table 4.7) and the fluorescence generated from the hydrolysis of this substrate (10252) was almost 4-times as high as that resulting from 4-MU-GAL hydrolysis (2836). The differences in fluorescence were less pronounced after 18 h incubation, although the average fluorescence generated upon hydrolysis of 4-MU-GAL was still lower to that of most of the novel coumarin ß-galactosidase substrates, with the exception of 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside. The second commercially available substrate, 7-hydroxycoumarin-4-acetic acid-β-D-galactoside, gave poor fluorescence at 6 h and a reasonable value at 18 h, as for 4-MU-GAL.

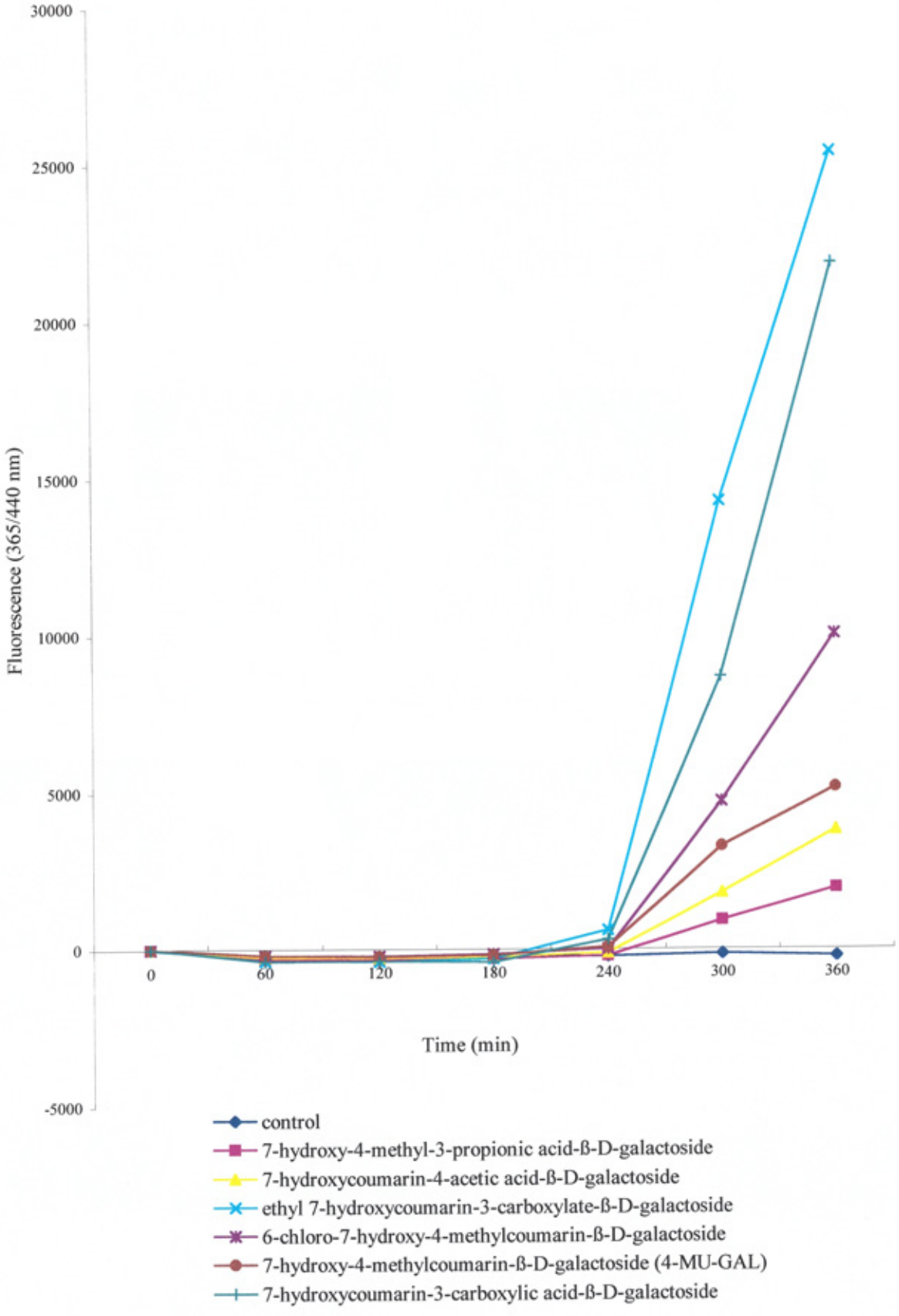
**Table 4.7: Average data from 12 coliform organisms showing (i) the effect of each coumarinic galactoside at 0.5 mmol $l^{-1}$ on coliform growth at 6 h and 18 h, and (ii) the fluorescence generated as a result of hydrolysis of the substrates by bacterial ß-galactosidase activity at 6 h and 18 h**

| Fluorogenic substrate in BHI broth | Relative growth at 6 h* (% control) | Relative growth at 18 h* (% control) | Average fluorescence at 6 h (relative units) | Average fluorescence at 18 h (relative units) |
|---|---|---|---|---|
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 97 | 100 | 300 | 13357 |
| Ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside (EHC-GAL) | 95 | 100 | 10252 | 30861 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 100 | 99 | 2267 | 32546 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 100 | 100 | 6388 | 28113 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside (4-MU-GAL) | 90 | 95 | 2836 | 25794 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 92 | 100 | 858 | 29894 |
| Control (growth medium) | 100 | 100 | - | - |

* based on absorbance increase at 690 nm

Figure 4.7 shows representative fluorescence data generated by the hydrolysis of six coumarinic galactosides by *C. freundii* (FRHCFR2) over 6 h incubation. These results are typical of those obtained for all coliform strains (see Appendix 4.5). While a number of coumarinic galactosides showed evidence of hydrolysis from 240 minutes onwards, the highest values are always observed for EHC-GAL making this the most sensitive derivative for a time-based assay method. Overall, for all of the 12 coliform organisms used in this investigation, EHC-GAL yielded maximal fluorescence upon hydrolysis, with an average fluorescence value more than 4-fold that of 4-MU-GAL after 6 h incubation. Although 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside generated the maximum average fluorescence of the ß-galactosidase substrates tested after 18 h incubation, it was not selected for application in a MPN assay due to the relatively low fluorescence generated after 6 h incubation, in contrast to EHC-GAL (Table 4.7 and Figure 4.7). Consequently, EHC-GAL was selected for testing in a MPN assay format, in a direct comparison with 4-MU-GAL and the standard US recommended medium (Anon., 2000), as described in Chapter 5.

**Figure 4.7: Fluorescence generated from the hydrolysis of a range of coumarinic galactosides (0.5 mmol $l^{-1}$) by *Citrobacter freundii* (FRHCFR2) in mMLSB.**



**Evaluation of fluorogenic glucuronide substrates**

Four of the coumarin molecules were derivatised to form glucuronide substrates (see Table 4.4). ß-D-glucuronide substrates were all used at an equivalent concentration of 0.5 mmol $l^{-1}$ to assess their fluorescence and any potential inhibitory effect on bacterial growth. As before, a table was compiled (Table 4.8) showing averaged values for percentage growth of the six *E. coli* strains, based on changes in absorbance readings at 690 nm after 6 h incubation and overnight incubation (18 h). The results show that after 18 h incubation none of the four newly synthesised coumarinic glucuronides were inhibitory to bacterial growth (see Appendix 4.6); only one substrate, ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-glucuronide, did not show an identical increase in absorbance to that of the growth control with the average increase in absorbance in the presence of the substrate being 98% that of the growth control. At 6 h however, it appears that one of the novel substrates, methyl 7-hydroxycoumarin-3-carboxylate-ß-D-glucuronide, inhibited bacterial growth to a substantial extent and so would not be suitable for a rapid detection system. This same substrate was non-inhibitory after 18 h incubation suggesting bacterial growth was slowed but not inhibited completely. The data also shows that 4-MU-GUR exhibited no growth inhibition whatsoever after both 6 h and 18 h incubation, this is in contrast to the slight inhibitory effects observed with 4-MU-GAL (Table 4.7).

Table 4.8 also details the average fluorescence generated upon hydrolysis of the substrates by the organisms under test and shows that certain glucuronide substrates generated substantially more fluorescence at 6 h than 4-MU-GUR upon hydrolysis (see Appendix 4.7). The fluorogen 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-glucuronide

generated the maximum fluorescence of the coumarinic glucuronides after 6 h incubation (9302); the fluorescence generated by hydrolysis of this novel substrate was more than three times the fluorescence generated upon hydrolysis of 4-MU-GUR (2810). The substrate which generated the least fluorescence upon hydrolysis was ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-glucuronide (EHC-GUR); this was even more pronounced at 18 h than at 6 h. The fluorescence generated upon hydrolysis of this substrate after 18 h incubation was less than one-fifth of the other substrates, in contrast to EHC-GAL which generated the maximum fluorescence of the coumarinic galactosides. The fluorescence generated upon hydrolysis of 4-MU-GUR compared well to that of most of the novel substrates after 18 h incubation, in contrast to the results for the coumarinic galactosides (Table 4.7).

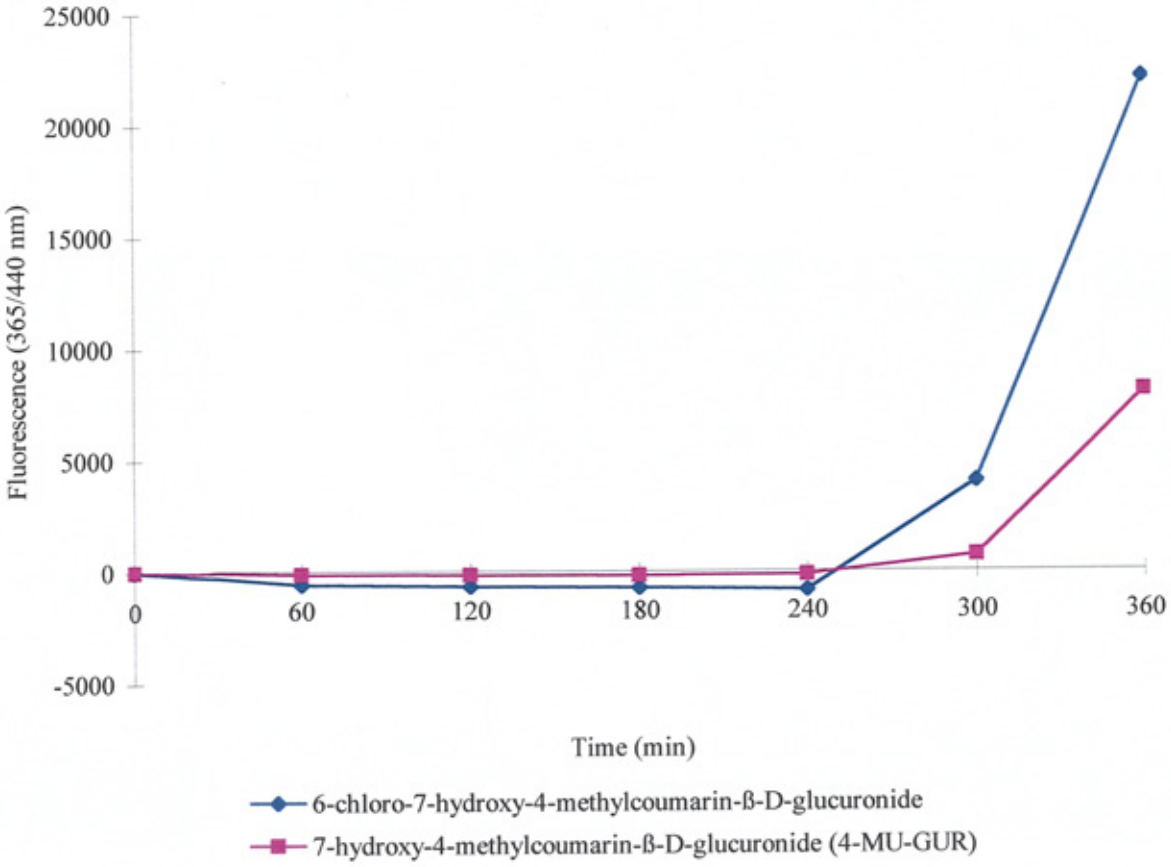
**Table 4.8: Average data from six *E. coli* strains showing (i) the effect of each coumarinic glucuronide at 0.5 mmol $l^{-1}$ on growth, and (ii) the fluorescence generated as a result of hydrolysis of the substrates by bacterial ß-glucuronidase activity at 6 h and 18 h**

| Fluorogenic substrate in BHI broth | Relative growth at 6 h* (% control) | Relative growth at 18 h* (% control) | Average fluorescence at 6 h (relative units) | Average fluorescence at 18 h (relative units) |
|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-glucuronide | 92 | 98 | 1770 | 4920 |
| benzyl 7-hydroxycoumarin-3-carboxylate-ß-D-glucuronide | 96 | 100 | 5271 | 25146 |
| methyl 7-hydroxycoumarin-3-caboxylate-β-D-glucuronide | 47 | 100 | 148 | 25383 |
| 6-chloro-7-hydroxy-4-methylcoumarin-β-D-glucuronide | 100 | 100 | 9302 | 29975 |
| 7-hydroxy-4-methylcoumarin-ß-D-glucuronide (4-MU-GUR) | 100 | 100 | 2810 | 29927 |
| Control (growth medium) | 100 | 100 | - | - |

* based on absorbance increase at 690 nm

Figure 4.8 illustrates the difference in fluorescence generated over 6 h resulting from the hydrolysis of 4-MU-GUR and 6-chloro-7-hydroxy-4-methylcoumarin-β-D-glucuronide by *E. coli* (FRHECO8); data for other strains is in Appendix 4.7. After 6 h incubation at 37°C (360 min) the fluorescence generated by the novel substrate was more than double (22059) that exhibited by released 4-MU (8015). This difference was even more pronounced at 300 min (5 h) where the fluorescence generated by 6-chloro-7-hydroxy-4-methylcoumarin-β-D-glucuronide (3997) is greater than four times that exhibited by 4-MU-GUR (695). This data suggests that a strong positive signal could be observed more rapidly if this novel substrate was used in a test medium as opposed to the commercially available coumarin-based ß-glucuronidase substrate.

**Figure 4.8: Fluorescence generated from the hydrolysis of two coumarin ß-glucuronides (0.5 mmol $l^{-1}$) by *Escherichia coli* (FRHECO08) in mMLSB.**



Fluorogenic substrates based on 4-MU have been widely used for both ß-galactosidase and ß-glucuronidase detection and coliform/*E. coli* testing of water samples (Covert *et al.*, 1992). Brenner *et al.*, (1993) evaluated 4-MU-GAL in a selective medium for *E. coli* and coliforms, termed MI agar, and compared counts to those on m-ENDO medium and two other media selective for *E. coli* (m-TEC agar and nutrient agar supplemented with 4-MU-GUR) using a membrane filtration method with natural and simulated samples containing coliforms and *E. coli*. Statistical analysis showed results for total coliform recovery were significantly different (0.05 significance level) with the new medium recovering up to 1.8 times as many total coliforms as m-ENDO. No statistically significant difference was observed between counts of *E. coli* on the new medium (MI agar) and those on m-TEC medium, although both media isolated significantly more *E. coli* than nutrient agar supplemented with 4-MU-GUR. This work was extended by Brenner *et al.* (1996) using chlorine-damaged organisms. Again the new medium (MI agar) was found to recover more target organisms, have less background flora and was better able to recover chlorine-damaged target organisms than conventional standard US media.

George *et al.* (2000) used a modified version of a method first described by Fiksdal *et al.* (1994). This involved membrane filtration of water samples, followed by incubation of the filters in a buffered solution containing 4-MU-GAL. The method is particularly rapid as it does not require a cultivation step. Filters were placed in this solution and then maintained at 37°C in a shaking water bath for 30 minutes. Every 5 minutes an aliquot was removed from the wash solution and placed into a quartz cell containing sodium hydroxide (1 mol $l^{-1}$) and the fluorescence measured (362/445 nm). The authors also repeated the assay using 4-MU-GUR. These detection methods were

compared with standard plate counts on lactose agar with Tergitol (Merck, Germany). George *et al.* (2000), found that the rapid enzyme technique permitted the detection of 'active but nonculturable' coliforms and *E. coli* within 30 minutes, and it was suggested that these tests could prove to be useful for monitoring sewage pollution in natural waters. However, as there is no selective growth step false-positive organisms could affect the assay, and as the pH of samples is increased to greater than 10 for assay samples cannot be subcultured for confirmation.

These studies give an indication of the interest generated by fluorescent enzyme substrates based on coumarin core molecules and the potential they may have in a range of applications to detect coliforms rapidly, without the need for confirmatory tests. A disadvantage of substrates based on 4-MU is that the maximum fluorescence of the reaction product requires an alkaline pH, since the $pK_a$ of 4-MU is around 7.8 (Goodwin and Kavanagh, 1950). Fluorescence is substantially quenched at pH levels below the $pK_a$ of the fluorophore, resulting in lower than optimal signals under most reaction conditions (Gee *et al.*, 1999). As a result an alkalinisation step may be required in fluorogenic assays based on 4-MU, usually involving the addition of concentrated alkali to the growth medium following enzymatic hydrolysis of the substrate, to obtain a pH greater than 10 and to give maximal fluorescence from this fluorophore (e.g. George *et al.*, 2000). In the present study, derivitisation of the 4-MU core molecule at various positions resulted in shifted fluorescence-pH curves, enabling high fluorescence at more acidic pH values, as shown in Figure 4.5. Such fluorescent characteristics offer the twin advantages of firstly, not requiring an alkalinisation step and secondly, potentially generating a larger signal for the assay of ß-galactosidase or ß-glucuronidase activity in a non-destructive continuous assay format.

Coliforms, including *E. coli*, generally survive in drinking water for up to 12 weeks, depending on environmental conditions such as water temperature, the presence of other microflora and exposure to solar ultraviolet radiation (Edberg *et al.*, 2000). During this time, they will become subjected to environmental stresses and may not respond as favourably as laboratory-grown cultures when incubated on selective media. McFeters *et al.* (1986) suggested that sub-lethal injury resulting from disinfectants might be responsible for reduced coliform counts under selective conditions. Their findings demonstrated that a high percentage (96.5%) of the coliforms present in chlorinated water were injured and were not detected when grown on m-ENDO immediately after filtration. Furthermore, a high percentage (78.0%) of samples with coliforms on a less selective medium (m-T7 agar) failed to yield colonies on m-ENDO.

The ability of enzyme detection methods based on 4-MU to recover stressed organisms has also been investigated; some studies have observed unacceptable levels of false-negative *E. coli* determinations in treated water systems (Clark *et al.*, 1991; Covert *et al.*, 1992). Factors such as these increase the potential applications of a substrate with a lower inhibitory effect than 4-MU, especially as the findings of LeChevallier *et al.* (1985) suggest that waterborne pathogenic bacteria are more resistant to injury by chlorine than similarly exposed coliforms; their study showed *Yersinia enterocolitica*, *Salmonella typhimurium* and *Shigella* spp. had lower susceptibilities to chlorine injury than enterotoxigenic *E. coli* and various members of the coliform group in the test system used. LeChevallier *et al.* (1985) suggested that the indicator bacteria may show more than 90% injury when the pathogens are not affected. The results of the present investigation with non-stressed coliforms suggest that the novel galactoside

and glucuronide substrates described here offer the potential for more effective enumeration of these organisms. This is due to the fact that the core molecules released upon hydrolysis are measurably less inhibitory to coliform growth than 4-MU, released when 4-MU-GAL and 4-MU-GUR are hydrolysed. Such differences may be further enhanced when the target organisms are sub-lethally injured, either under laboratory conditions (see Chapter 2 for UV-A damaged organisms or Chapter 3 for chlorine-damaged organisms) or in environmental water samples (see Chapter 5).

The results shown in this chapter demonstrate that a selection of coumarin-based ß-galactosidase and ß-glucuronidase substrates have been synthesised which mostly proved to be as good as, and in several cases better than, the commercially available alternatives 4-MU-GAL and 4-MU-GUR. These novel substrates generated high levels of fluorescence when hydrolysed by bacterial enzymes and proved to be non-inhibitory to bacterial growth when present both as the substrate and as the core molecule released upon hydrolysis. Differences in the pH-fluorescence curves of the substituted coumarin molecules offers the potential for more rapid tests, or for reduced substrate concentrations in test media. These aspects are further considered in Chapter 5.

# CHAPTER FIVE

## Application of novel fluorogens for coliform detection in a rapid 'most probable number' assay

## Background

As previously discussed in Chapter 1, there are two standard methods for the enumeration of total coliforms and faecal coliforms from water. The multiple tube method provides an MPN value; it is well accepted that the existing MPN procedure is a method of low precision resulting from the limited number of tubes which can be used for each sample. Most laboratories tend to use three or five sets of replicate tubes and, unless a large number of samples are examined, the precision of MPN is low (Belieff and Mary, 1993). The membrane filtration (MF) technique enumerates coliforms on the surface of a membrane placed on an agar plate or a broth-soaked pad, providing a CFU count per 100 ml (Geissler *et al.*, 2000). Both of the tranditional methods are based on the ability of coliforms to produce acid from lactose-based media. Conventional culture techniques such as these are easy to perform and provide a relatively cheap way to enumerate faecal indicator bacteria for water quality assessment (Sartory and Watkins, 1999). The key problems with both methods are firstly, the time taken to get a confirmed result and secondly, the inability of the procedures to differentiate between coliforms and *E. coli* without further tests (Gleeson and Gray, 1997). If cultural approaches are to have a future in enumeration and demonstration of viability, methods must be developed that significantly reduce the time needed to obtain a confirmed result, and increase the sensitivity of detection (Sartory and Watkins, 1999).

The Colilert QuantiTray system (Idexx, USA) is an expanded MPN procedure that employs the chromogen *ortho*-nitophenol-ß-D-galactoside (ONPG) and the fluorogen

4-MU-GUR for the detection of ß-galactosidase and ß-glucuronidase respectively. Unlike traditional MPN techniques there is no requirement for confirmatory tests after the initial observation of a positive result. To perform the test, sample water is added to powdered formula in a tray which is then incubated. No equipment other than an incubator and UV-lamp is required (Edberg *et al.*, 1988a, 1988b). Evaluations in the UK by Fricker *et al.* (1997) indicated that the system may be a suitable alternative to the current UK membrane filtration reference method (Anon., 1994), offering confirmed counts for *E. coli* and coliforms within 18 h incubation and being less labour-intensive (Sartory and Watkins, 1999). In the US, evaluations by Edberg *et al.* (1989) indicated that Colilert was a sensitive and specific method for the simultaneous detection of coliforms and *E. coli* in drinking water, and that it was compatible with the Safe Drinking Water Act regulations (Edberg *et al.*, 1989).

A limitation recognised with the Colilert system was that injured coliforms can give a weaker yellow colour making interpretation difficult. The availability of computer software and fluorescent microtitre plate readers allows rapid and automated screening of microtitre trays containing fluorescent MPN assays. A miniaturised multiple-tube procedure has been developed (Hernandez *et al.*, 1991) which utilises a 96-well microtitre plate for an MPN assay using the commercially available fluorogenic compound 4-MU-glucuronide (4-MU-GUR). Water samples are added to wells containing growth medium and the substrate, with the number of rows inoculated depending on the presumptive contamination level of the sample. This assay, which is specific for *E. coli*, provides results within 18 h. A particular advantage of using a miniaturised system is that, due to the small volumes of sample inoculated into each microtitre well (100µl), the number of replicates can be increased dramatically from

those used in standard methods. This has the effect of narrowing the 95% confidence limits and increasing the precision involved in statistically estimating the MPN. This chapter looks at a modification of this technique and incorporates the fluorogenic ß-galactosidase substrates evaluated in Chapter 4.

## Experimental objective

1) To evaluate the performance of a broth assay, based on a miniaturised MPN format, incorporating a novel fluorogenic substrate, in comparison to the commercially available 4-MU-GAL and standard methods.

# Materials and methods

Unless otherwise stated all materials and equipment was as described in previous chapters. Lauryl Tryptose Broth (LTB, Anon., 1994) was prepared from its constituents as follows: lactose, 5 g; tryptose, 20 g; dipotassium hydrogen phosphate, 2.75 g; potassium dihydrogen phosphate, 2.75 g; sodium chloride, 5 g; sodium lauryl sulphate, 0.1 g; bromocresol purple, 0.01 g; all of which were obtained from Sigma-Aldrich Chemical Company Ltd., Poole, UK.

**Application of galactosides to a miniaturised MPN assay format**

As previously discussed, this was based on a miniaturised MPN procedure (Hernandez *et al.*, 1991) which utilises a 96-well microtitre plate for the MPN assay. All initial work was carried out using the Biolite F1 fluorescent plate reader. A trial was carried out with 5 fluorogenic galactoside substrates and three coliform organisms *K. pneumoniae* (NCTC 10896); *C. freundii* (NCTC 9750) and *E. coli* (FRHECO2), in order to estimate the time required to achieve an accurate MPN estimation. Standard LTB was also included in the assay for comparison. In all cases, strains were cultivated on Columbia agar for 18 h at 37°C followed by inoculation into 10 ml BHI broth for a further 18 h at 37°C. MPN dilutions were then prepared, based on initial bacterial population estimates of $\approx 5 \times 10^9$ cfu $ml^{-1}$ in BHI broth; plate counts were taken prior to the MPN assay to confirm bacterial numbers. The galactoside substrates were prepared as follows: 4-MU-GAL (1 mmol $l^{-1}$, 0.34 g $l^{-1}$); EHC-GAL (1 mmol $l^{-1}$, 0.40 g $l^{-1}$); methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside (1 mmol $l^{-1}$,

0.37 g $l^{-1}$); 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside (1 mmol $l^{-1}$, 0.37 g $l^{-1}$); 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside (1 mmol $l^{-1}$, 0.37 g $l^{-1}$).

Duplicate microtitre trays were prepared which each contained two replicates of each organism dilution, of which there were eight, resulting in a total of four replicates per organism dilution per medium type. In all cases LTB was modified (mLTB) to contain no lactose or bromocresol purple and prepared at double strength. All fluorogenic substrate broths incorporated IPTG (at 60 mg $l^{-1}$) and were prepared as described earlier (Chapter 4). Each substrate/IPTG broth was added to microtitre trays in 50 µl aliquots, followed by the addition of an equal volume of diluted bacterial suspension, resulting in final concentrations of 0.5 mmol $l^{-1}$ substrate and 30 mg $l^{-1}$ IPTG. Appropriate substrate-free and bacteria-free controls were included in each microtitre tray. Standard LTB (prepared at double strength to include lactose and bromocresol purple) was used in the investigation to compare with MPN values achieved in the fluorogenic assay. Absorbance was read every 2 h for 10h, fluorescence was read hourly for 4 h and then every 30 minutes for a further 6 h. After this 10 h analysis all trays were incubated at 37°C for a further 14 h, at which point 24 h readings were recorded for both absorbance and fluorescence, and then re-incubated for a further 24 h to attain 48 h data. It should be noted that MPN counts were recorded visually for standard LTB at 24 h and 48 h only, as recommended in US standard methods (Anon., 2000).

The novel substrate which generated the most promising results was then included in a modified version of the procedure used by Hernandez *et al.* (1991), based on

three doubling dilutions of low organism counts with 32 replicates per dilution, to achieve MPN values for a total of 10 coliform organisms diluted to low inoculum densities (< 200 cfu $ml^{-1}$). EHC-GAL (1 mmol $l^{-1}$, 0.40 g $l^{-1}$) was directly compared with 4-MU-GAL (1 mmol $l^{-1}$, 0.34 g $l^{-1}$); both substrate-broths incorporated IPTG (60 mg $l^{-1}$) and were prepared as described earlier (Chapter 4, i.e. at double strength). Standard LTB was again used in the investigation to compare with MPN values achieved in the fluorogenic assay. *E. coli* NCIMB 10213, *C. freundii* NCTC 9750, *Ent. cloacae* NCTC 11936, *Ent. aerogenes* NCIMB 10102 and *K. pneumoniae* NCTC 10896 were included in this assay, along with one wild strain of each species (FRHECO2, FRHCFR2, FRHECL2, FRHEAE2, FRHKPN2 respectively). A range of doubling dilutions was prepared for each organism in sterile water to attain estimated counts in the order of ≈ 200, 100 and 50 cfu $ml^{-1}$. Aliquots of 100 µl of organism were added to 100 µl of each double-strength mLTB. A total of 32 replicates of each dilution for each organism were included in this assay and the dilution series gave predicted counts in the approximate order of ≈ 20, 10 and 5 cfu per microtitre well, with substrate present at 0.5 mmol $l^{-1}$.

Inoculated microtitre plates were incubated for a total of 11 h with shaking at 37°C. Fluorescence (365/440 nm) was read at time zero and again at 6 h, then reading half hourly for a further 5 h. Trays were then incubated overnight (37°C), reading fluorescence at 24 h to give maximum counts. Microtitre trays containing standard LTB were incubated without shaking at 37°C, giving preliminary results at 24 h: trays were re-incubated for a further 24 hours to give maximum MPN values.

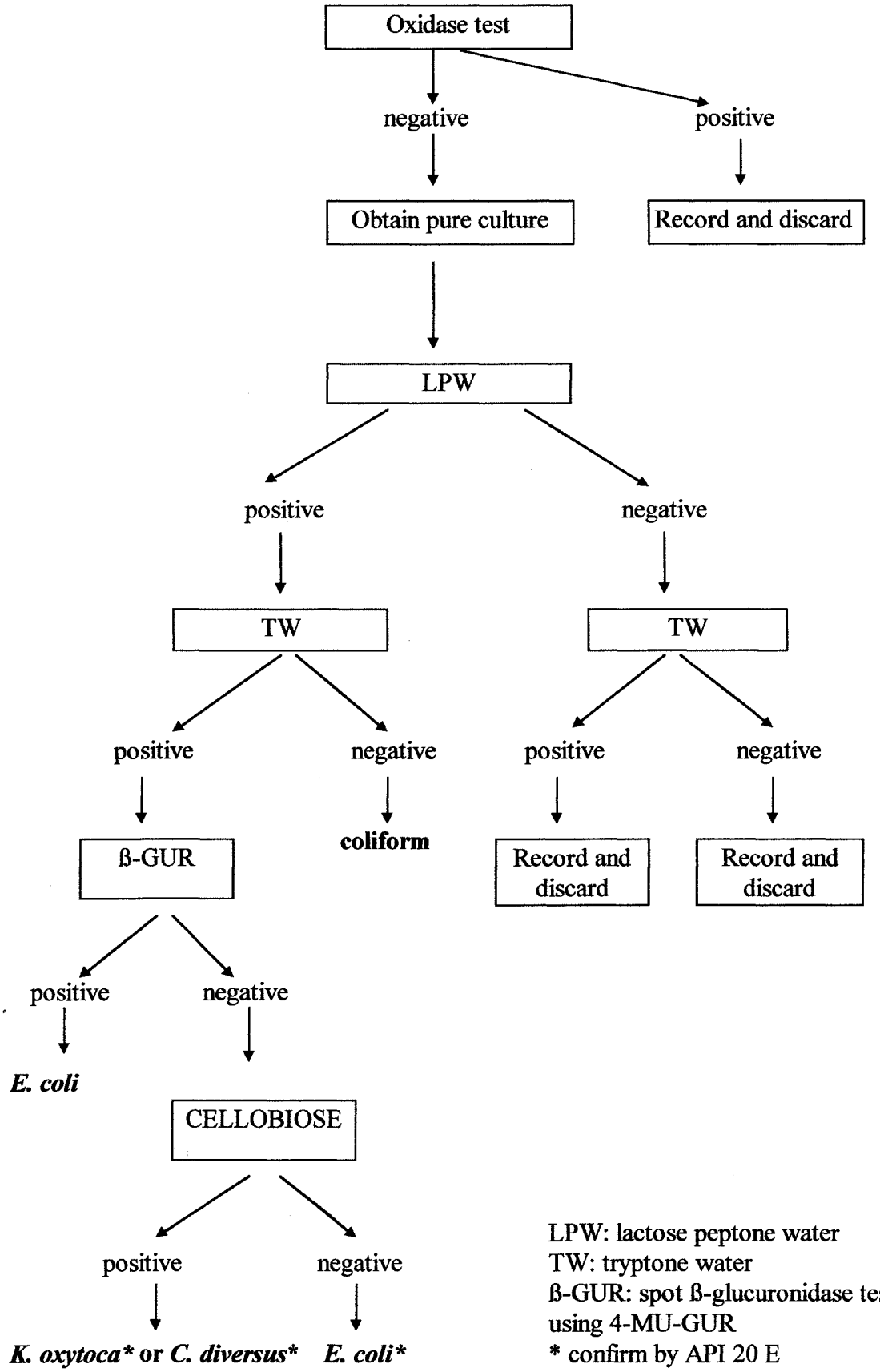
**Miniaturised MPN assay of water samples**

The above study incorporating 4-MU-GAL, EHC-GAL and standard LTB was repeated with water samples obtained from Hebburn Water Treatment plant (Analytical Environmental Services, North Tyneside). Each water sample came from a different stage in the water treatment process (see Figure 1.1); raw sewage, settled stage, filtration stage and final treated sample. A kinetic assay was carried out using the Biolite F2 fluorescent plate reader, over a period of 18 h, incorporating a series of 6 decimal dilutions each with 7 replicates. A distilled water control was included for each substrate broth (6 replicates). The microtitre template allowed both fluorogenic substrates to be tested in one tray to avoid discrepancies in temperature, etc.. Again mLTB containing substrate was prepared at double strength, and diluted in the well with test water. Beginning with 'neat' sample, decimal dilutions were carried out in the microtitre tray ($10^0$ - $10^5$) to achieve a sample volume of 90 µl to which was added 90 µl of the relevant broth (final volume of 180 µl). The tray containing standard LTB was incubated for 18 hours at 37°C. To avoid dehydration, the tray to be assayed over 18 h in the plate reader was sealed with sellotape and each well was pin-pricked to maintain aerobic conditions. Whilst preparing this tray the fluorimeter was 'pre-heated' to a chamber temperature of 37°C, and this temperature was maintained throughout the 18 h assay.

As this study was now dealing with real water samples, presumptive positive wells needed to be confirmed before an actual coliform MPN could be achieved. A summary of the confirmatory tests used in this investigation is shown in Figure 5.1. All presumptive positive wells were sub-cultured onto a Columbia blood agar plate and

incubated at 37°C for 18 h. If a mixed culture was obtained after initial subculture, all colony types underwent an oxidase test; only those which were oxidase-negative were subcultured for further confirmation. Once pure, oxidase-negative colonies were inoculated into lactose peptone water (LPW) and tryptone water (TW) in microtitre wells (100 µl) and incubated at 37°C for 18 h. As in previous tests the production of indole was detected by the addition of Kovac's reagent. If colonies were indole-positive a spot ß-glucuronidase test was carried out using 4-MU-GUR; colonies which were indole-positive, ß-glucuronidase-negative underwent a cellobiose test to see if they were ß-glucuronidase-negative *E. coli*. If colonies were indole-positive, ß-glucuronidase-negative, cellobiose-positive they were confirmed as *Klebsiella oxytoca* or *Citrobacter diversus* (see Figure 5.1).

**Figure 5.1: Summary of confirmatory tests for MPN assay**




LPW: lactose peptone water
TW: tryptone water
ß-GUR: spot ß-glucuronidase test using 4-MU-GUR
* confirm by API 20 E

# Results and Discussion

## Application of galactosides to miniaturised MPN assay format

The purpose of the initial study was to establish if the MPN achieved within 10 hours was a reasonable estimation of the actual count. For example, if the MPN achieved after 10 h was the same as the MPN at 24 h, it would suggest that a 10 h kinetic assay would be a useful way of estimating MPN values more rapidly. Furthermore, if the actual MPN was achieved in 10 h when using 4-MU-GAL, it may be achieved more rapidly with a novel fluorogenic substrate which generates a much stronger fluorescent signal (Chapter 4). Additionally the study would establish whether the MPN values achieved with fluorogenic substrates were comparable to those achieved with standard LTB.

A formula was written in Microsoft Excel® which was applied to the data to indicate when each well became positive. It involved comparing each reading with the previous reading to see if an increase in fluorescence had occurred, and whether this increase was sufficient enough to indicate a 'positive' well; i.e. not just a small fluctuation in fluorescence but a significant increase which indicated that a ß-galactosidase-positive coliform was present in that well and the substrate was beginning to be hydrolysed. The MPN data obtained were then analysed using a computer-assisted method for determining population counts using Microsoft Excel® (Briones and Reichardt, 1999). The formulae used for deriving MPN estimates involved Microsoft Excel® and the Solver tool, the range of relevant dilutions could be broadened as the formula was

expandable. This was followed by statistical analysis using a t-test to see if there was a significant statistical difference (95% level) between MPN values obtained using standard methods (LTB) and those obtained using the novel fluorogenic assay.

Figures 5.2 - 5.4 show the MPN values achieved by *E. coli* (FRHECO2), *K. pneumoniae* (NCTC 10896) and *C. freundii* (NCTC 9750) when assayed over 10 h (600 min) in mLTB containing the fluorogenic ß-galactosidase substrates (see Appendices 5.1 and 5.2). Figure 5.5 shows the MPN counts at 24 h for the five fluorogenic substrates and standard LTB. EHC-GAL performed consistently well for all of the three organisms included in this assay, particularly *E. coli* (Figure 5.2). The MPN of this organism was achieved at 540 min (9 h) with both EHC-GAL and 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside, furthermore, the MPN was 37.7% higher than that achieved with 4-MU-GAL for the same organism. The lowest MPN was achieved with 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside for all test organisms. An encouraging point to note with this organism is that the MPN count at 600 min, or in the case of EHC-GAL at 540 min, is the same as the MPN count at 24 h (Figure 5.5).

Similarly to *E. coli*, the MPN counts for *K. pneumoniae* at 600 min were the same as those at 24 h, with the exception of one substrate (7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside). The MPN acheived by 4-MU-GAL at 600 min was 17.6% that of the MPN with EHC-GAL, this difference remains at 24 h. 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside achieves an MPN count more than twice that of 4-MU-GAL at 600 min, however the maximum count with this substrate was not recorded until the 24 h reading (Figure 5.5). The data suggest that the strain of

*C. freundii* included in this assay was relatively slow-growing in comparison to the other organisms (Figure 5.4). The MPN counts achieved with this organism were relatively low at 600 min for all substrates, although the highest MPN was achieved with EHC-GAL. Again the lowest MPN count at 600 min was achieved by 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside, and interestingly, 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside, which with the other organisms had performed comparably to EHC-GAL (Figure 5.5).

Figure 5.5 (see Appendix 5.3) suggests that EHC-GAL is the most suitable substrate for a rapid MPN assay as it achieves the highest MPN counts at 24 h, and in addition, the maximum MPN counts were reached within 600 min, for two of the three organisms included in this study. For *C. freundii*, the organism for which the maximum MPN count was not reached within 600 min, the count at 600 min was highest with this substrate indicating that this substrate would give a more accurate representation of organism numbers at 600 min than with any of the other fluorogenic substrates including 4-MU-GAL. This is a very encouraging factor in the development of a rapid MPN assay as it suggests that a reasonably accurate quantitative estimate of overall coliform presence could be achieved within 10 h. The data obtained for LTB was fairly poor, in the case of two of the organisms (*E. coli* FRHECO3 and *K. pneumoniae* NCTC 10896) the MPN count for LTB was worse than that of mLTB with 4-MU-GAL and for one organism (*C. freundii* NCTC 9750) the lowest MPN count was achieved with LTB.

**Figure 5.2: MPN values achieved by *Escherichia coli* (FRHECO2) within a 10 h assay when grown in five different fluorogenic versions of mLTB.**

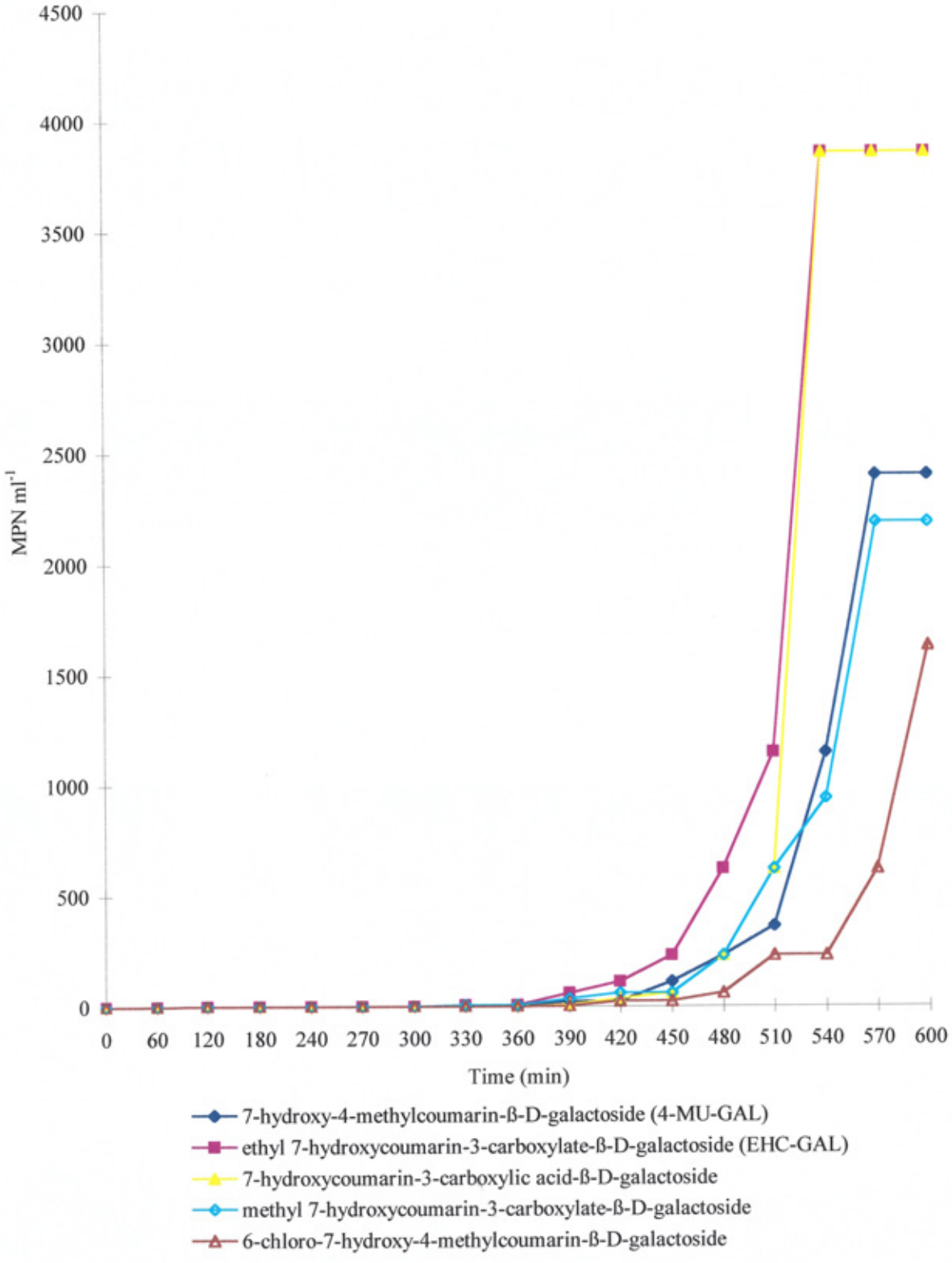


**Figure 5.3: MPN values achieved by *Klebsiella pneumoniae* (NCTC 10896) within a 10 h assay when grown in five different fluorogenic versions of mLTB.**

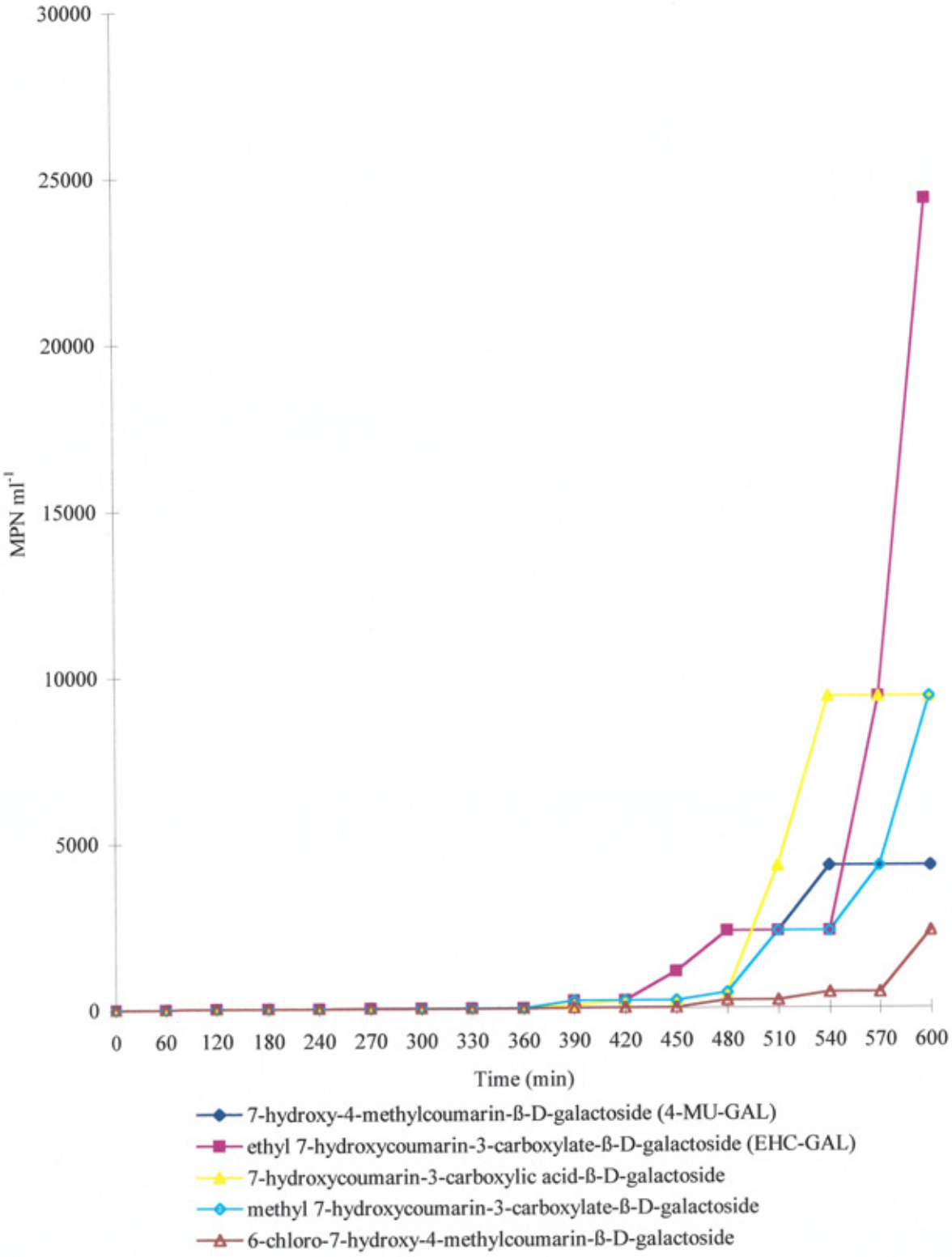


**Figure 5.4: MPN values achieved by *Citrobacter freundii* (NCTC 9750) within a 10 h assay when grown in five different fluorogenic versions of mLTB.**

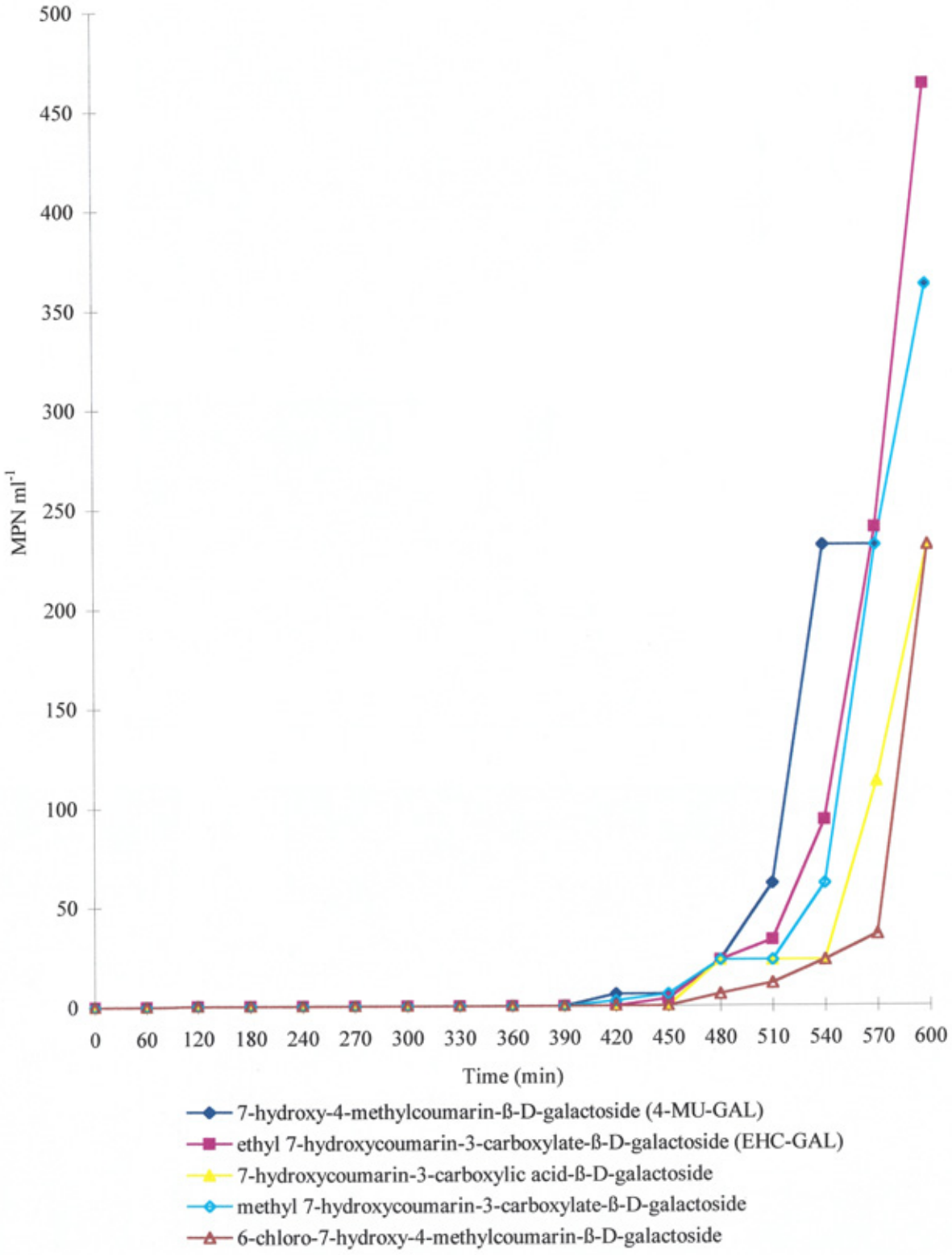


**Figure 5.5: Comparison of the MPN values acheived with three coliform organisms with five fluorogenic mLTB and standard LTB after 24 h incubation.**

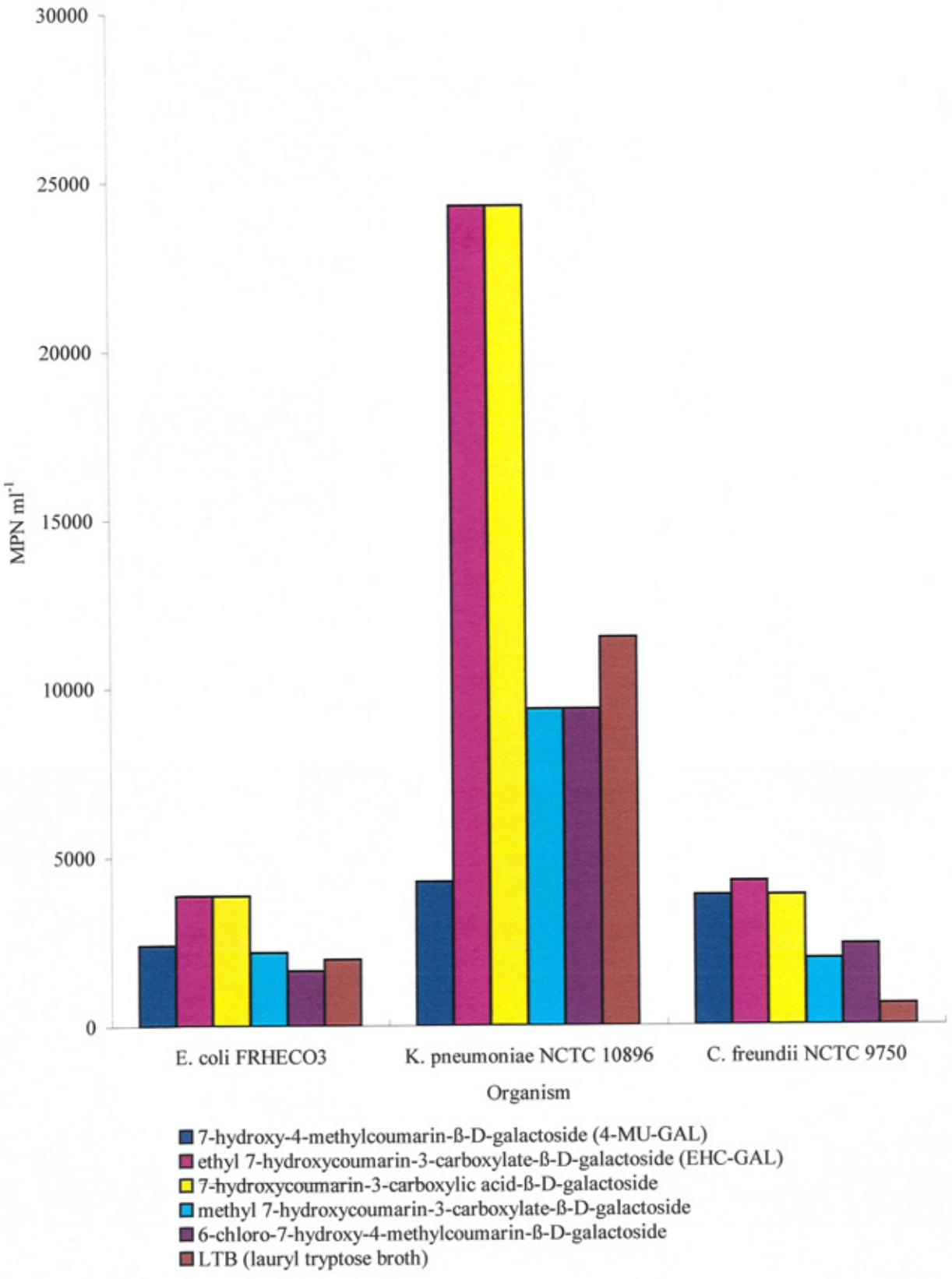


Figure 5.6 shows a comparison of MPN values achieved with 10 different coliforms in 2 fluorogenic modifications of mLTB after 11 h incubation (see Appendices 5.4 and 5.5). The incubation time was increased to 11 h as the previous study indicated that some organisms (e.g. *C. freundii*) did not show comparable MPN counts at 10 h to those obtained at 24 h. This illustrates that with 8 of the 10 coliform organisms used in this study mLTB containing 4-MU-GAL generated lower MPN values after 11 h incubation than in mLTB containing EHC-GAL, indicating the potential of the latter medium for detecting low numbers of target organisms more quickly than existing methodologies. However, for 2 coliforms, the MPN value was highest in mLTB containing 4-MU-GAL. When increases in MPN values over the incubation period were compared for each substrate it was apparent that a positive MPN value ($\geq 1$ CFU/ml) was reached with EHC-GAL on average 30 minutes earlier than with 4-MU-GAL, as shown for all test strains in Figure 5.7. This figure indicates the first time at which a positive signal was confirmed, i.e. the time at which an increase in fluorescence had been generated which was greater than a fluctuation in fluorescence. However, four strains showed no difference in the time taken to give a positive value in 4-MU-GAL and EHC-GAL, while one strain (*E. coli* NCIMB 10213) gave no positive result for 4-MU-GAL within the experimental period (see Figure 5.6).

**Figure 5.6: Comparison of the MPN values acheived with 5 NCTC strains and 5 wild strains of various coliforms with 2 modifications of Lauryl Tryptose Broth (mLTB) after 660 min (11 h) incubation at 37°C**

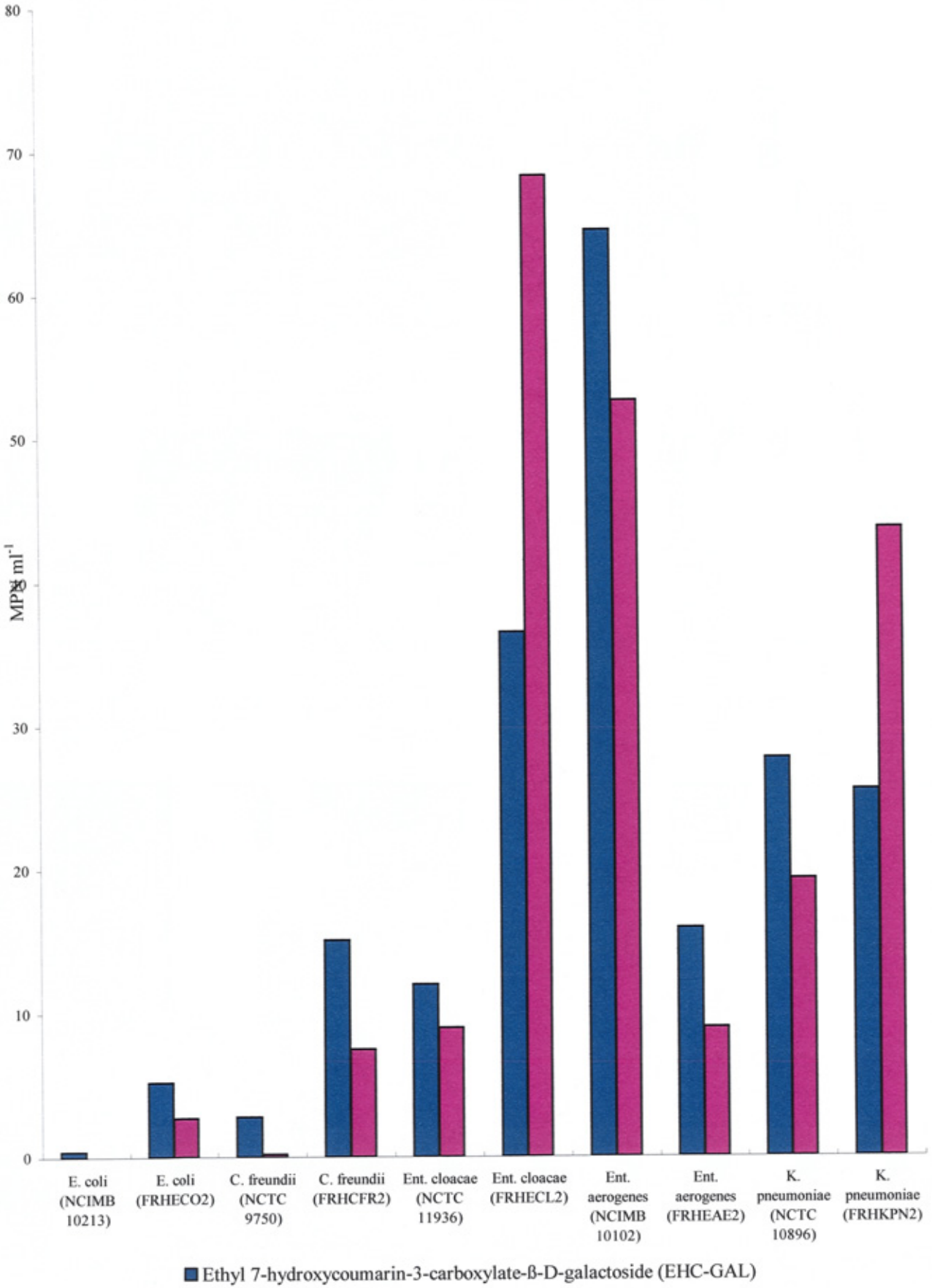


**Figure 5.7: Time required for the test organisms to achieve a positive MPN (> 1 CFU/ml) value in two fluorogenic media.**

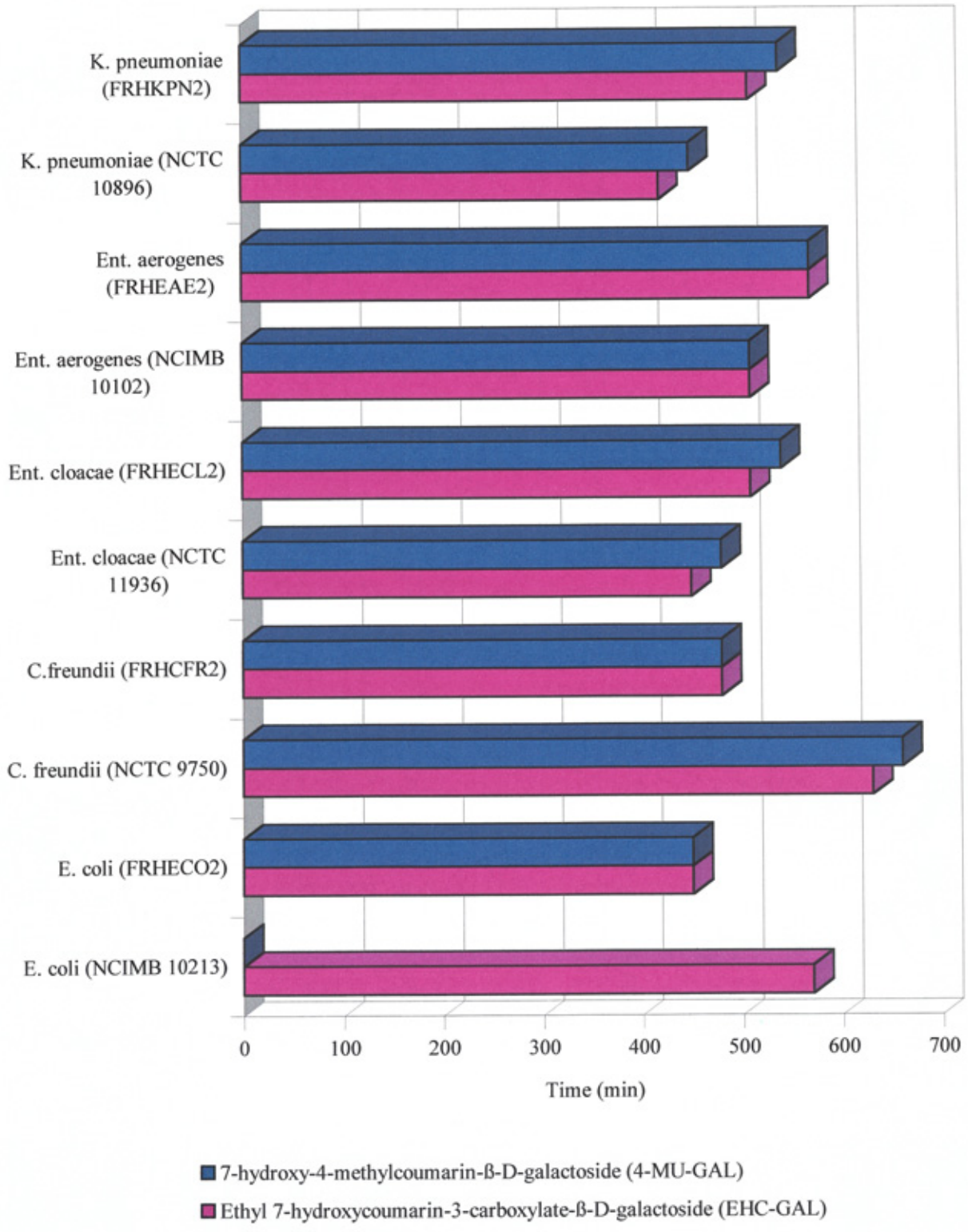


Figure 5.8 gives a typical result for a single strain, showing the increase in MPN $ml^{-1}$ for *K. pneumoniae* (NCTC 10896) over the time period when samples were assayed half hourly (see Appendices 5.4 and 5.5). The final MPN $ml^{-1}$ value of 27.7 was reached by EHC-GAL at 630 min, at this same time the MPN $ml^{-1}$ of 4-MU-GAL was 17.4. The maximum MPN (24 h) achieved by the organism when grown in mLTB containing EHC-GAL was 27.7, the count attained at 630 min, whereas at 24 h the MPN value of *K. pneumoniae* grown in mLTB containing 4-MU-GAL was still rising and was over 18% lower (at 22.5) than that attained in EHC-GAL. A positive MPN value (≥ 1 CFU/ml) was detected at the same time (450 min) in the presence of both substrates for this particular organism, but the increase in MPN counts was faster in the presence of EHC-GAL than in 4-MU-GAL.

**Figure 5.8: A comparison of MPN values of *Klebsiella pneumoniae* (NCTC 10896) over 24 h incubation in the presence of 4-MU-GAL and EHC-GAL.**

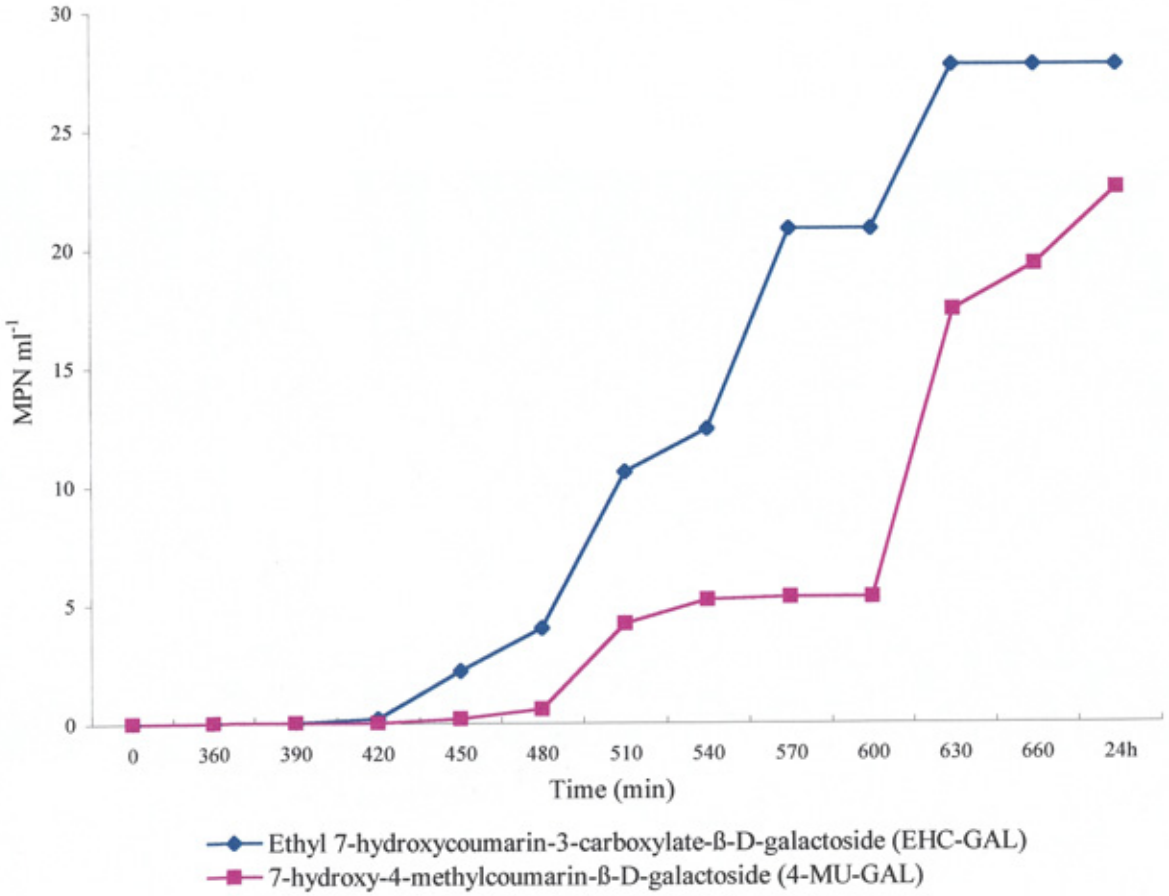
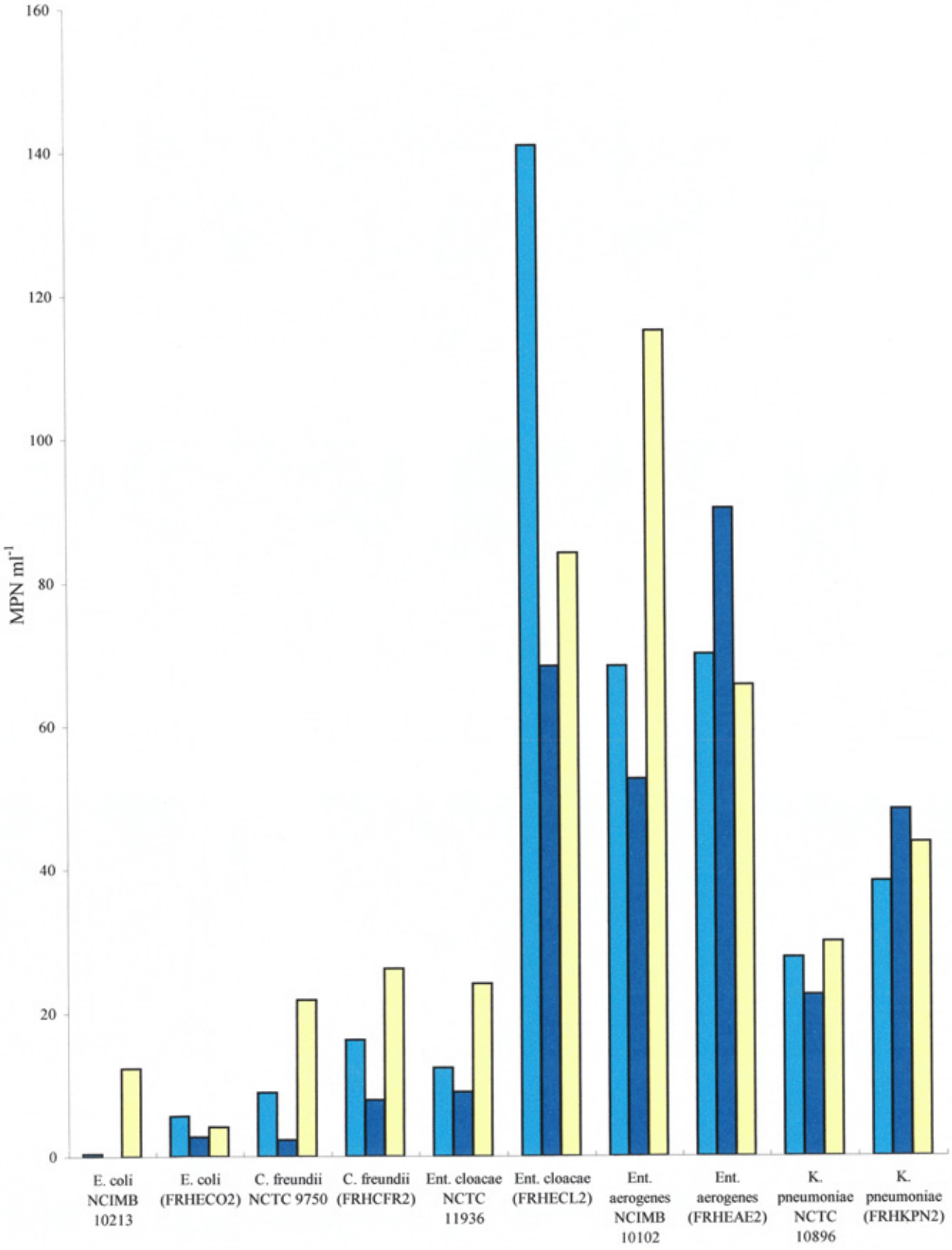


Figure 5.9 shows the MPN values achieved after 24 h using both fluorogenic substrates in comparison with those achieved using standard LTB (see Appendix 5.5). As with the 11 h data (Figure 5.6), the novel substrate EHC-GAL generated higher MPN values than 4-MU-GAL with 8 of the 10 organisms included in the assay and lower values for the remaining 2 coliforms. Although the highest MPN values were achieved in standard LTB for six of the organisms, the advantage of having an automated assay system that can record at what point each well became positive, determine the final MPN count, and avoid extensive confirmatory tests suggests that the novel fluorogenic media may provide a suitable alternative to the existing methodology.

The data for Figure 5.9 were analysed statistically. Comparisons of the difference between each fluorogenic substrate in mLTB medium and the standard LTB medium using a one-sided paired t-test (to see whether each novel medium was significantly lower than LTB) gave a P value of 0.056 for the comparison between mLTB containing 4-MU-GAL and standard LTB only. The P value for the equivalent comparison between mLTB containing EHC-GAL and standard LTB was 0.32. Although the P value for the mLTB plus 4-MU-GAL/standard LTB comparison is just above that required to determine a statistically significant difference, the results give an indication that MPN values obtained using 4-MU-GAL as a fluorogenic substrate may be somewhat lower than those obtained with US standard LTB medium. This may be of concern where 4-MU-GAL is used in rapid assay format, though the analysis warrants repeating with a larger data set, to see whether a statistically significant difference emerges.

**Figure 5.9: Comparison of the MPN values acheived with 5 NCTC strains and 5 wild strains of various coliforms with unmodified and 2 modifications of standard lauryl tryptose broth (LTB) after 24 h incubation at 37°C.**



**MPN miniaturised MPN assay of water samples**

The water samples for this part of the study were samples taken from different stages in the water treatment process; raw sewage, settled stage, filtration stage and final treated sample. Figure 5.10a illustrates the increase in MPN $ml^{-1}$ for the raw sewage sample over the 18 h assay period when grown in mLTB containing EHC-GAL and mLTB containing 4-MU-GAL. The figure shows both unconfirmed MPN counts and confirmed MPN counts for this sample, Figure 5.10b shows only the confirmed counts. Figures 5.11a and b, 5.12a and b, and 5.13a and b, are for settled, filtered and final treated sewage samples respectively. Appendices 5.9 - 5.10 show all raw data for these figures.

**Figure 5.10a: MPN counts from a 'raw' sewage sample over an 18 h assay when grown in mLTB containing EHC-GAL and mLTB containing 4-MU-GAL; figure shows both unconfirmed and confirmed counts.**

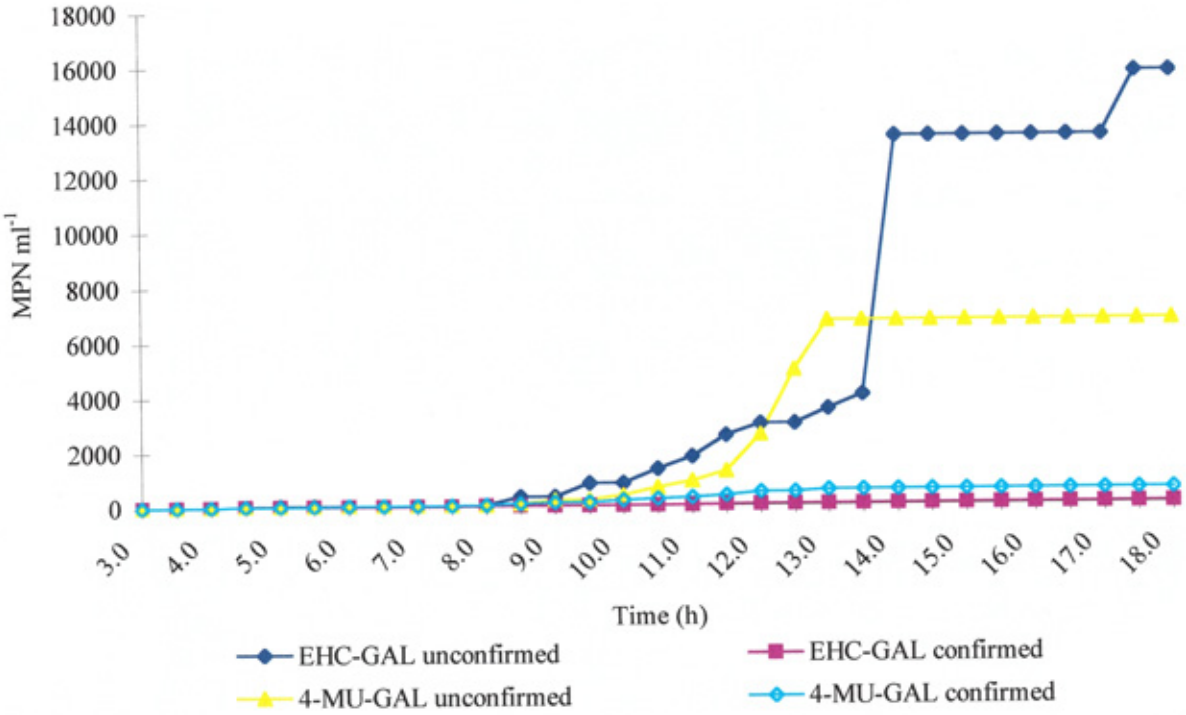



**Figure 5.10b: MPN counts from a 'raw' sewage sample over an 18 h assay when grown in mLTB containing EHC-GAL and mLTB containing 4-MU-GAL; confirmed counts only.**

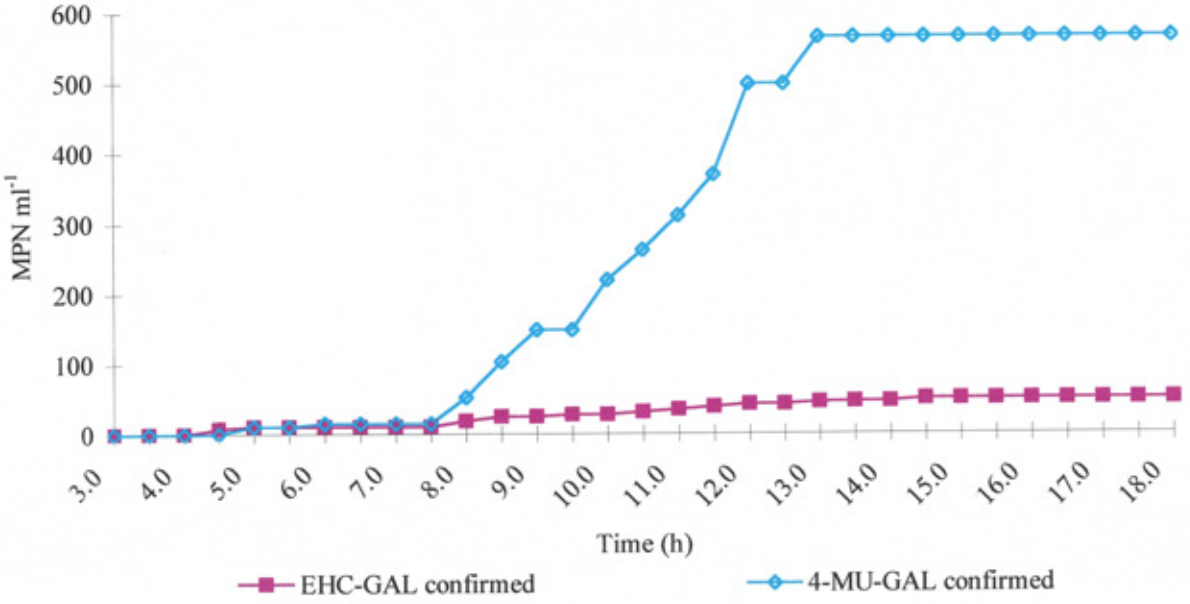


**Figure 5.11a: MPN counts from a 'settled' sewage sample over an 18 h assay when grown in mLTB containing EHC-GAL and mLTB containing 4-MU-GAL; figure shows both unconfirmed and confirmed counts.**

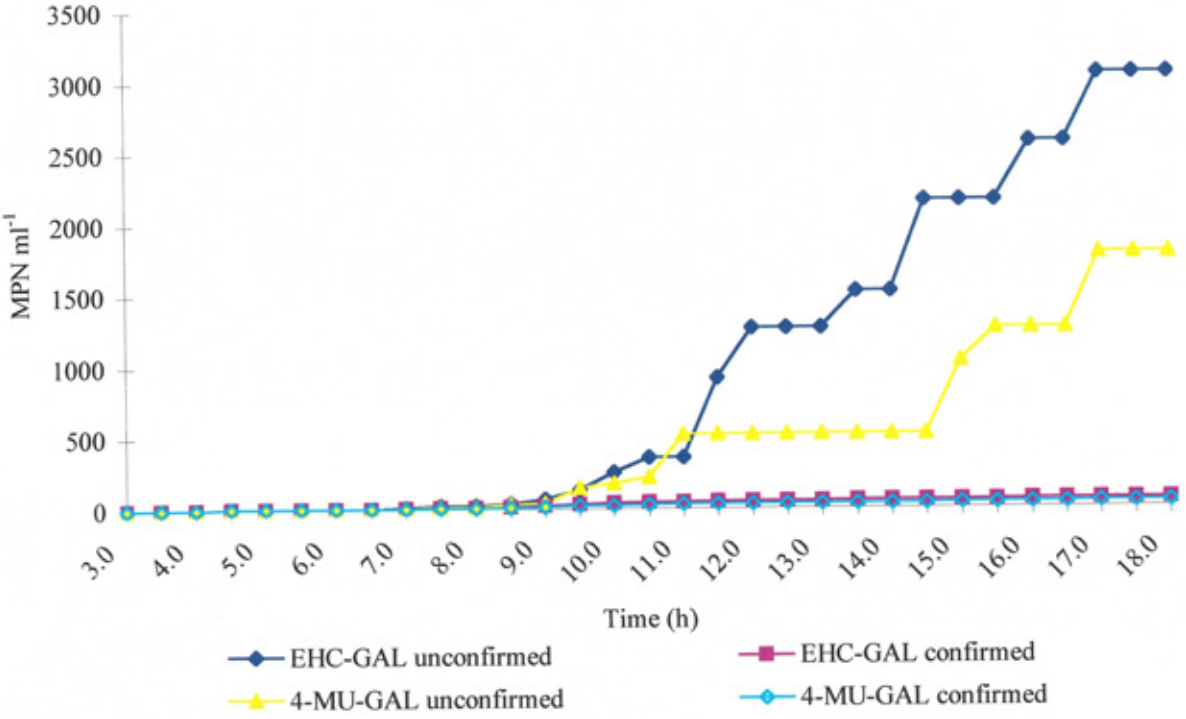



**Figure 5.11b: MPN counts from a 'settled' sewage sample over an 18 h assay when grown in mLTB containing EHC-GAL and mLTB containing 4-MU-GAL; confirmed counts only.**

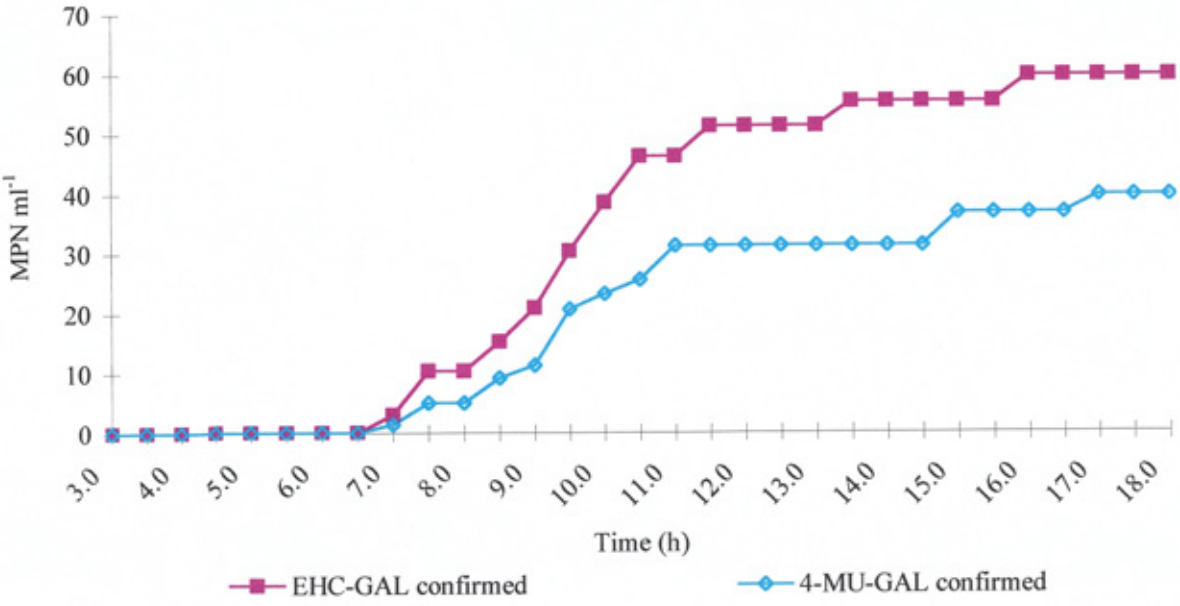


**Figure 5.12a: MPN counts from a 'filtered' sewage sample over an 18 h assay when grown in mLTB containing EHC-GAL and mLTB containing 4-MU-GAL; figure shows both unconfirmed and confirmed counts.**

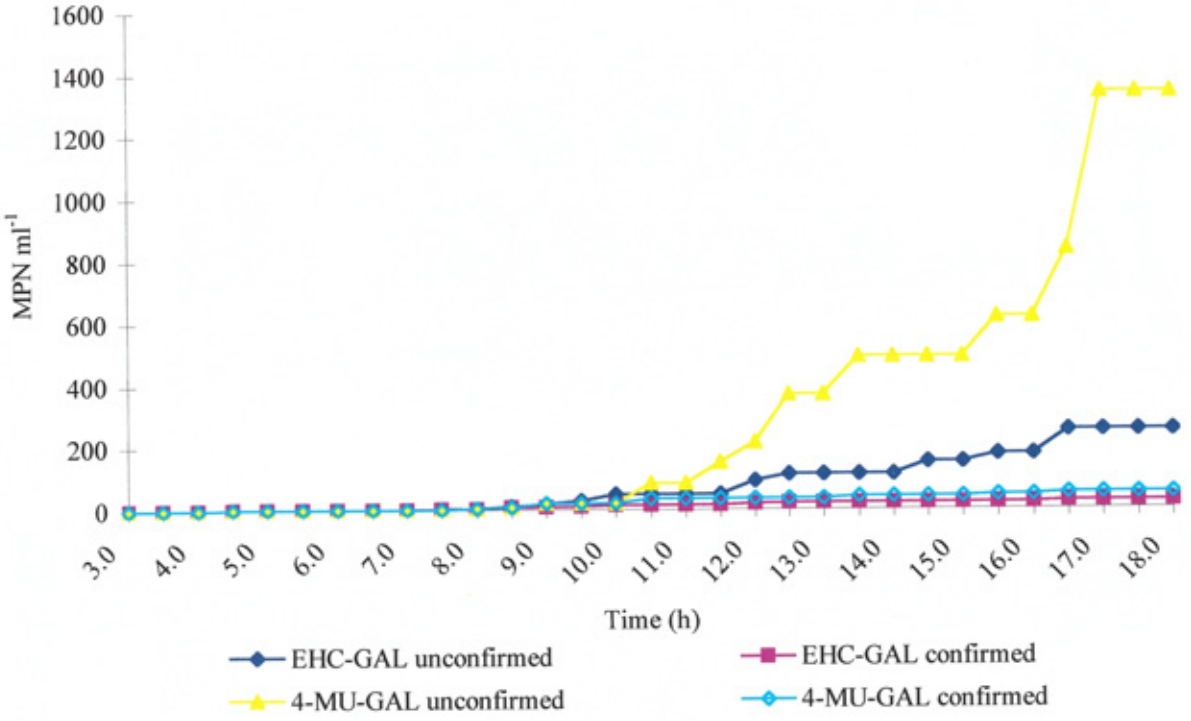



**Figure 5.12b: MPN counts from a 'filtered' sewage sample over an 18 h assay when grown in mLTB containing EHC-GAL and mLTB containing 4-MU-GAL; confirmed counts only.**

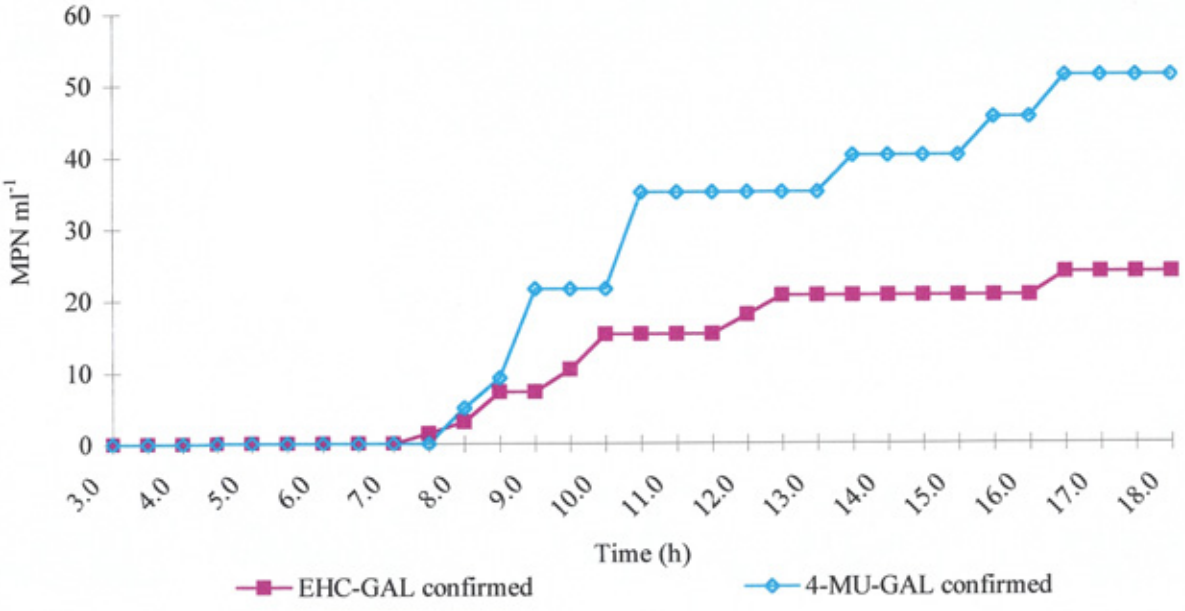


**Figure 5.13a: MPN counts from a 'final' sewage sample over an 18 h assay when grown in mLTB containing EHC-GAL and mLTB containing 4-MU-GAL; figure shows both unconfirmed and confirmed counts.**

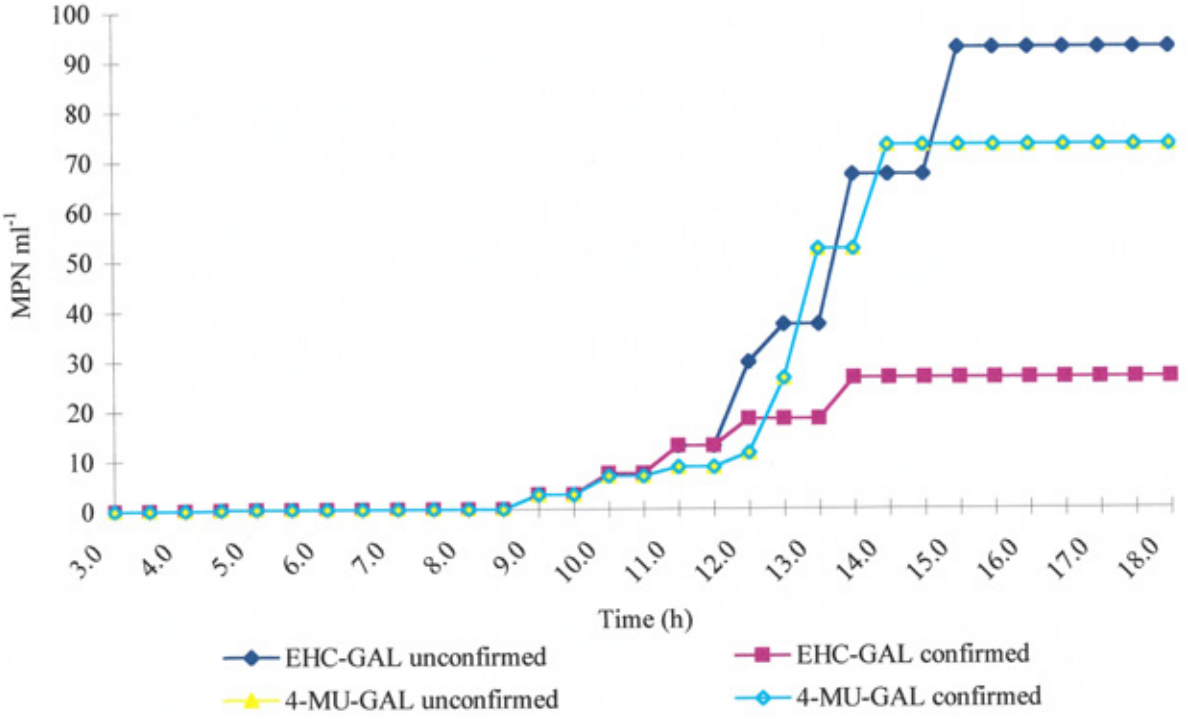
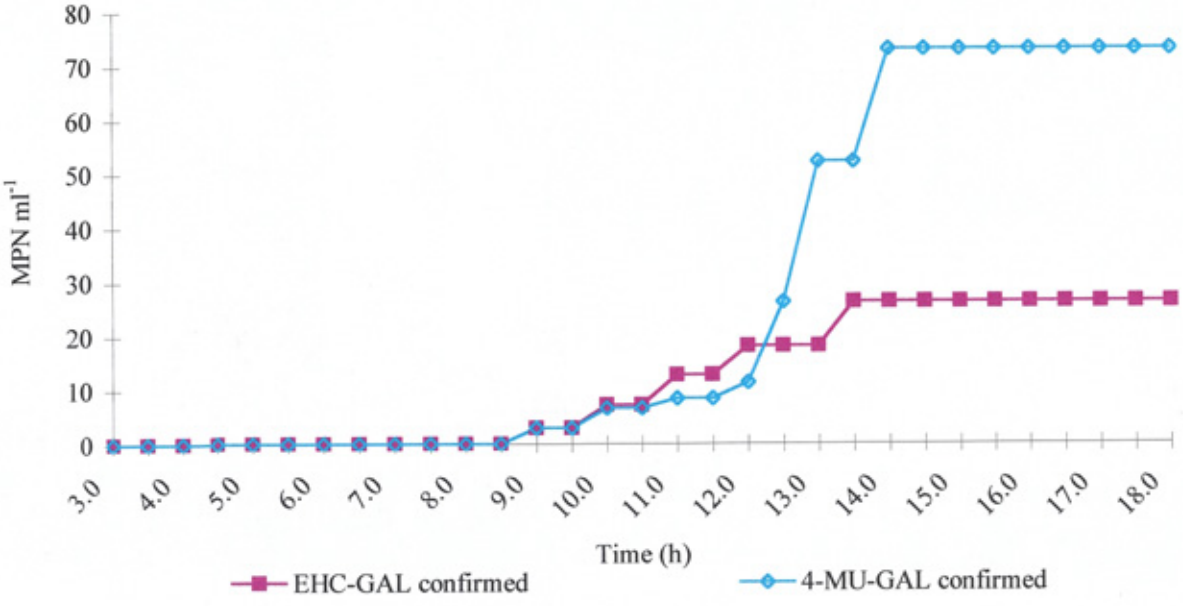



**Figure 5.13b: MPN counts from a 'final' sewage sample over an 18 h assay when grown in mLTB containing EHC-GAL and mLTB containing 4-MU-GAL; confirmed counts only.**



The figure sets 5.10 and 5.13 clearly show mLTB containing EHC-GAL generated the highest presumptive MPN values. However, after confirmatory testing, mLTB containing 4-MU-GAL exhibited the highest MPN values. In the case of the raw sewage sample (Figures 5.10a and 5.10b) the number of confirmed coliforms in mLTB containing EHC-GAL was 8.7% (49.0 MPN $ml^{-1}$) of the number of confirmed coliforms in mLTB containing 4-MU-GAL (564.1 MPN $ml^{-1}$). The difference was not as pronounced in the case of the final stage sewage sample where the number of confirmed coliforms in mLTB containing EHC-GAL was 35.9% (26.2 MPN $ml^{-1}$) of the number of confirmed coliforms in mLTB containing 4-MU-GAL (72.9 MPN $ml^{-1}$). These differences in the proportion of presumptive positive wells which confirmed to contain coliforms are fairly difficult to explain. One possible reason for this variation could be the heterogenicity of the samples, particularly with sewage samples from the early stages of processing. Raw sewage samples contain significant quantities of discrete particulate matter and faecal material making it difficult to obtain a homogenous sample for inoculation into microtitre wells. This problem is enhanced when dealing with such low volumes of sample (90 µl).

The data in Figure 5.11a and b show mLTB containing EHC-GAL generated the highest MPN $ml^{-1}$ counts both at the presumptive stage and after confirmation. The data illustrated in Figure 5.12a and b show the opposite, with mLTB containing 4-MU-GAL generating the highest MPN $ml^{-1}$ counts both at the presumptive stage and after confirmaion. As expected, the time taken for a well to become 'positive' increased as the inoculum decreased; for example, a confirmed coliform MPN count was observed at 4.5 h (EHC-GAL) and 5.0 h (4-MU-GAL) for the raw sewage sample, while for the settled sewage sample this increased to 7.0 h for both fluorogenic substrate media.

With the filtered sample this increased slightly to 7.5 h (EHC-GAL) and 8.0 h (4-MU-GAL) and for the final sample a confirmed MPN count was achieved by both mLTB containing EHC-GAL and mLTB containing 4-MU-GAL at 9.0 h (Figure 5.13b).

An observation from the data for the raw sewage sample is that with mLTB containing 4-MU-GAL, of the 25 presumptive positive wells at 18 h a total of 49 colonial variants were subcultured, 19 of which were oxidase-positive (38.8%). In contrast, with mLTB containing EHC-GAL, of the 29 presumptive positive wells at 18 h a total of 61 colonial variants were isolated, 34 of which were oxidase-positive (55.7%). It is possible that this could indicate that the novel substrate is slightly more sensitive than 4-MU-GAL and is therefore being hydrolysed more readily by 'contaminating' weak ß-galactosidase producers which are oxidase-positive, such as strains of *Aeromonas* spp. It is also possible that because the novel substrate, and core molecule released upon hydrolysis, are slightly less inhibitory to microbial growth than 4-MU-GAL, the broth containing EHC-GAL is more likely to grow a greater density of organisms. This could account for the greater number of colonial variants observed in mLTB plus EHC-GAL. It was previously thought that only high levels of *Aeromonas* sp. could generate false-positive reactions in fluorogenic assays (Cowburn *et al.*, 1994), however, more recent studies have indicated that very low levels of *Aeromonas* sp. can lead to false-positive results in fluorogenic media (Landre *et al.*, 1998). The increase in colony types would also make it more difficult to isolate pure cultures of organisms, including coliforms. It is therefore possible that because subcultures from wells containing EHC-GAL were more dense/mixed than those from wells containing 4-MU-GAL, coliforms were missed and therefore did not reach the confirmatory stages.

Table 5.1 shows the number of positive wells observed with each sample type in each fluorogenic medium and the number of colonial variants isolated. The table also lists the proportion of these isolates that were oxidase-positive. For three of the test samples the number of colonial variants isolated from mLTB containing EHC-GAL was higher than for mLTB containing 4-MU-GAL. Furthermore, for three of the test samples the proportion of colony types which were oxidase-positive was higher for the growth medium containing EHC-GAL, supporting the comments made in the preceding paragraph and indicating some of the possible limitations of the novel fluorogenic substrate.

**Table 5.1: Summary of the number of positive wells and the number of colonial variants they contained for each sample type.**

| Water sample | Fluorogenic substrate | Number of positive wells | Number of colonial variants | Number of oxidase-positive isolates | % of oxidase-positive isolates |
|---|---|---|---|---|---|
| Raw sewage sample | mLTB: 4-MU-GAL | 25 | 49 | 19 | 38.8 |
| | mLTB: EHC-GAL | 29 | 61 | 34 | 55.7 |
| Settled sewage sample | mLTB: 4-MU-GAL | 21 | 35 | 11 | 31.4 |
| | mLTB: EHC-GAL | 24 | 36 | 13 | 36.1 |
| Filtered sewage sample | mLTB: 4-MU-GAL | 23 | 39 | 11 | 28.2 |
| | mLTB: EHC-GAL | 16 | 31 | 17 | 54.8 |
| Final sewage sample | mLTB: 4-MU-GAL | 11 | 18 | 5 | 27.8 |
| | mLTB: EHC-GAL | 12 | 19 | 5 | 26.3 |

Figure 5.14a and 5.14b show the 18 h MPN $ml^{-1}$ values for all of the test media in this study (see Appendix 5.10). Once again the data show substantial differences in the proportion of presumptive positive wells which confirmed as having a coliform present. As with previous data it is unlikely that these differences are due to a particular medium performing better than another medium, and is more likely to be a result of inoculation of a non-homogenous sample. It is interesting to note that the largest differences in confirmed MPN values were observed with the two initial stage samples (raw and settle sewage), where the most particulate matter and faecal material would have been present.

**Figure 5.14a: Comparison of unconfirmed MPN values from four sewage waters taken from different stages in the treatment process in three modifications of LTB; mLTB with EHC-GAL, mLTB with 4-MU-GAL, and standard LTB after 18 h incubation at 37°C**

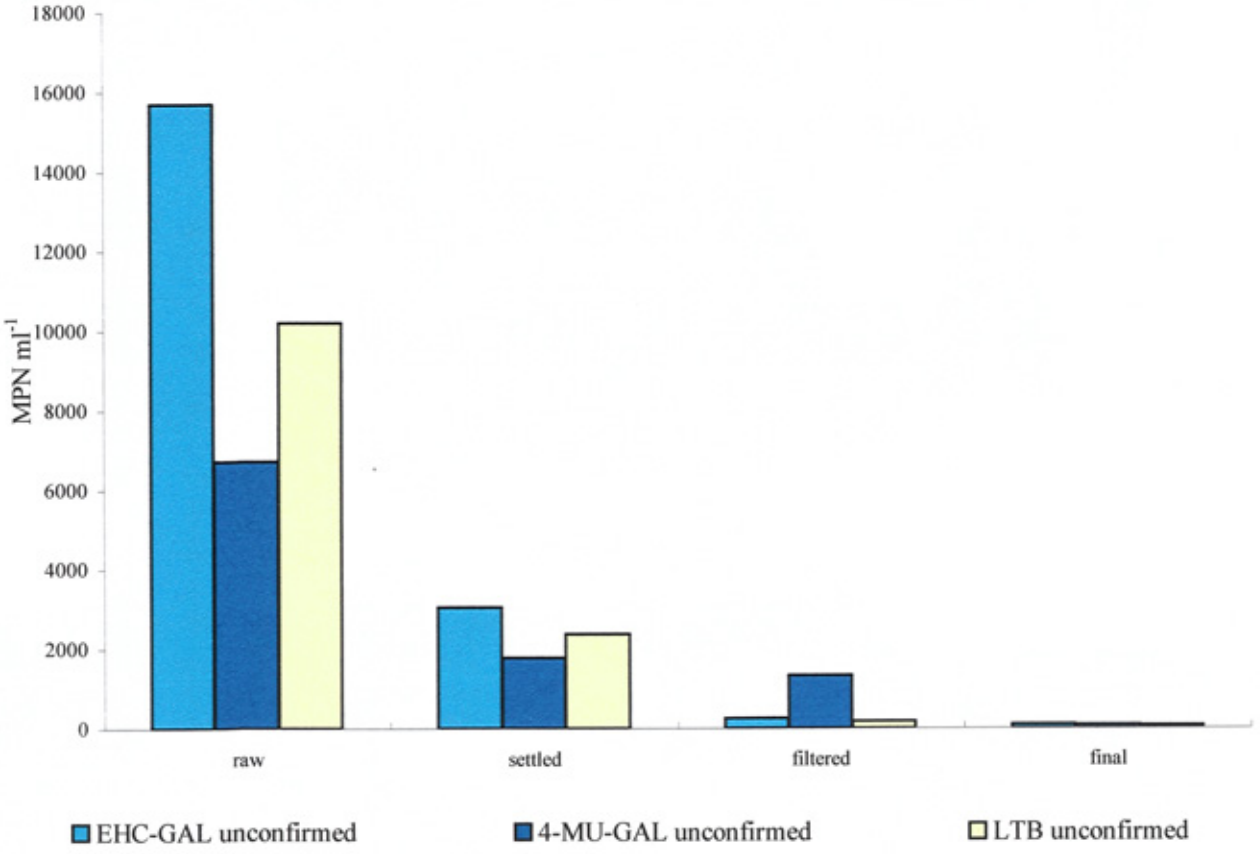
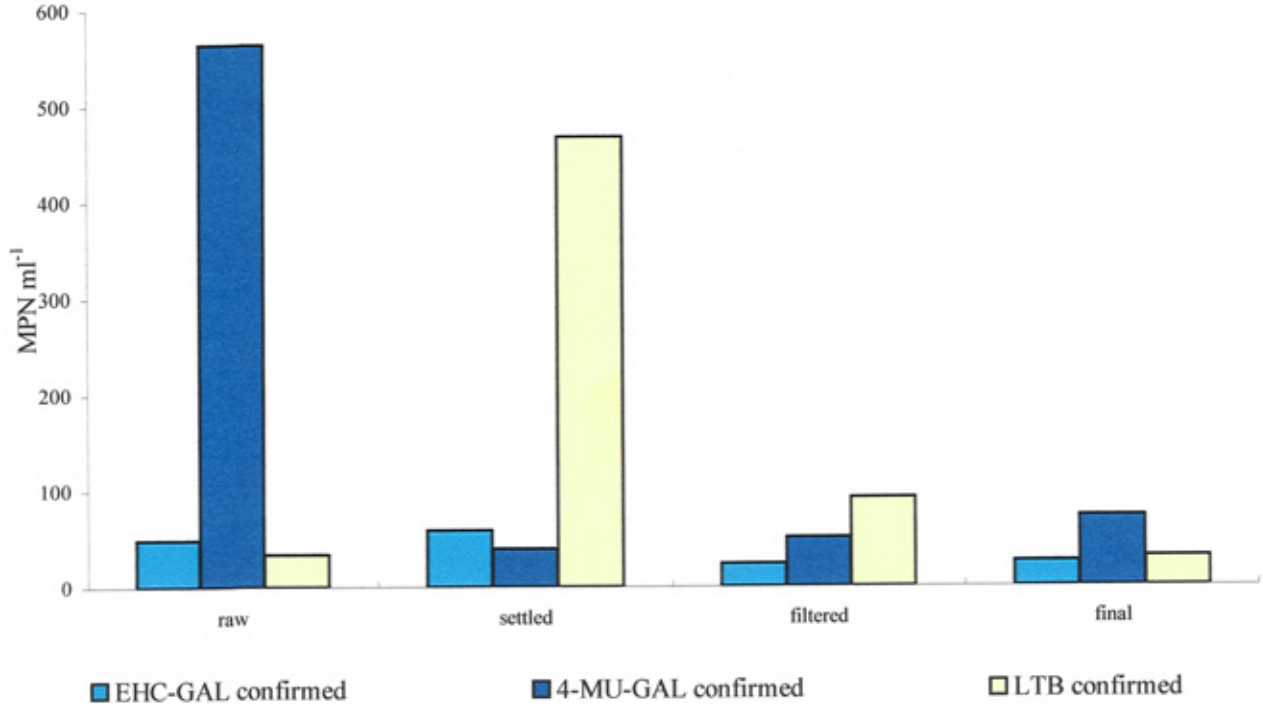



**Figure 5.14b: Comparison of confirmed MPN values from four sewage waters taken from different stages in the treatment process in three modifications of LTB; mLTB with EHC-GAL, mLTB with 4-MU-GAL, and standard LTB after 18 h incubation at 37°C**



One of the objectives of the MPN-based assay was to compare the rate at which coliform bacteria could be detected by monitoring hydrolysis of EHC-GAL in comparison with 4-MU-GAL. A study carried out by Park *et al.* (1995) looked at a fluorogenic MPN assay involving two modifications of LTB. The standard broth was compared to LTB supplemented with 4-MU-GAL and LTB supplemented with 4-MU-GUR. A total of 95 river and 153 reservoir water samples were tested, samples were serially diluted five times as described in standard methods (Anon., 1992) and inoculated into 10 ml of broth. Fluorescence was read at 2 h intervals ($4^{th}$ through $12^{th}$ h of incubation), for the standard assay the MPN value was recorded at 18 h and 24 h. The authors observed a significant increase in fluorescence by both fluorogenic substrate broth types at 12 h incubation, the preceding reading, at 10 h, showed an insignificant fluorescence reading which did not differ sufficiently from the growth control. In summary Park *et al.* (1995) observed that the length of incubation time required for the fluorogenic assay was reduced from 24 h to 12 h with the use of spectrophotometric assay. A similar study carried out by Clark and El-Shaarawi (1993) showed that fluorescence responses appeared more quickly than gas production and that fluorescence was detected earlier when incubated at $35^0$C than at 44.5°C. Sarhan and Foster (1991) looked at a membrane filtration assay containing 4-MU-GUR and were able to detect ß-glucuronidase activity by the development of fluorescent colonies within 7.5 h. The authors observed that the development of fluorescence was delayed when carbohydrates were incorporated into the medium. The miniaturised MPN assay described in Chapter 5 detected confirmed coliform counts as early as 4.5 h in the presence of EHC-GAL (5.0 h in the presence of 4-MU-GAL) with some

samples (see Figure 5.10b). The assay incorporated fluorogenic substrates into mLTB which did not contain the carbohydrate source lactose (5 g $l^{-1}$) which may have accelerated fluorescence generation.

The use of enzyme-specific substrates has significantly reduced the labour and time involved in processing water samples for coliform indicator bacteria. However, more meaningful protection of public health would be achieved if results of coliform and *E. coli* assays were available on the same day as the samples were collected, allowing prompt remedial action to be taken (Sartory & Watkins, 1999). Initial studies demonstrated that higher MPN values were achieved within 11 h incubation when using mLTB plus EHC-GAL for 8 of the 10 coliform organisms included in the trial. This indicated the potential of the substrate for use in a rapid assay. Furthermore, the significance of the MPN assay was the time taken to detect a positive well containing the target coliform organism, which might indicate a treatment failure in a drinking water sample, where a coliform count of zero would be expected. A fluorogenic detection system could indicate when a positive result (= "coliform present") was achieved, so that the relevant action could be taken, while the medium would then be further incubated to attain a quantitative result at 18 h, or 24 h, for example. The statistical analysis of the 24 h incubation data presented illustrated that the novel substrate EHC-GAL performed comparably to the US standard recommended medium, LTB.

When a study was performed with sewage samples as opposed to pure cultures of laboratory strains several potential problems were encountered. Firstly, the need for a homogenous sample was clear. The inoculation of a number of replicates of small

volumes of sample achieves one MPN value. In order to generate a reliable quantitative estimate of a sample a number of MPN values would need to be obtained so that an average could be established. Previous studies (Hernandez *et al.*, 1991) have suggested that the number of replicates should be increased, depending on the presumptive contamination level of the sample. Secondly, the fact that the novel substrate (EHC-GAL) initially appeared to be more sensitive and less inhibitory than 4-MU-GAL may not have been an advantage when using 'real' samples. Generally a higher number of colonial variants were observed in the medium containing the novel substrate, increasing the possibility that small numbers of coliforms were missed during the subculture process.

# CHAPTER SIX

## Discussion and further developments

Coliform analyses constitute by far the greatest proportion of the workload of the routine water microbiology laboratory: in the UK it has been estimated that over 5 million are carried out by the water industry each year (Sidorowicz and Whitmore, 1995). The detection of coliforms has traditionally been based on the production of acid and gas in lactose-based medium. For over half a century, diagnostic tests have been employed which are dependent on the possession of particular enzymes (James, 1994). A less selective medium for the recovery of coliforms would be advantageous, as it is well recognised that coliforms subjected to chlorine may become stressed, resulting in a reduced ability to grow on selective media (Camper and McFeters, 1979; LeChevallier and McFeters, 1985). Surface-active agents in media selective for coliform organisms are well-established, since bile salts were first recommended by MacConkey (1908). Bile salts lacked precise chemical definition so inter-batch variability could not be entirely eliminated and so were subsequently replaced by lauryl sulphate (Mallmann and Darby, 1941) and then Teepol solutions (Jameson and Emberley, 1956). Teepol solutions were less prone to such variability and a standard grade, Teepol 610, was used. This was discontinued in 1976 and so purified sodium lauryl sulphate was used as an alternative (Stanfield and Irving, 1981).

Failure to detect coliforms can lead to an overly optimistic estimate of the safety of a water sample (Calabrese and Bissonnette, 1990a). Consequently, a number of suggestions have been made to improve the detection and isolation of these bacteria. Wright (1984) suggested the inclusion of tryptose to increase the isolation of stressed organisms which are unable to grow in the presence of bile salts. The proposed medium gave higher counts of coliforms from faecal samples and comparable counts from contaminated water and food samples compared to standard media.

Subsequently, Calabrese and Bissonnette (1990a) proposed the addition of catalase or sodium pyruvate, or both, to growth media to enhance the growth of chlorine-stressed bacteria. These authors suggested that the addition of peroxide-degrading compounds to standard recovery media could improve the detection of coliforms. Sartory and Howard (1992) incorporated 0.05% (w/v) sodium pyruvate into their novel chromogenic agar for *E. coli* detection in drinking water samples. The medium was a modification of the standard UK membrane filtration medium (MLSB) and the authors reported increased detection of chorine-stressed coliforms.

The use of a less selective medium incorporating enzymatic substrates could potentially further increase recovery and reduce the need for extensive confirmatory tests, which are both time-consuming and costly. The coliform group possess the enzyme ß-galactosidase which is capable of hydrolysing a chromogenic or fluorogenic galactoside to obtain galactose for growth (Clesceri *et al.*, 1998). The use of media containing chromogenic or fluorogenic substrates for the enzymes ß-galactosidase and ß-glucuronidase for simultaneous detection of coliforms and *E.coli* is increasing (Fricker and Fricker, 1996). The present study employed the use of X-GAL, an indoxylic ß-galactosidase substrate which generates a blue colour upon hydrolysis, and CHE-GUR, a novel substrate which forms a chelate with iron upon hydrolysis, generating black colonies in a membrane filtration assay format. Initial work with the medium showed promising results; colonies were much easier to count due to the intense localisation of colour, recoveries of organisms were good, and the number of confirmatory tests would potentially be reduced. The results achieved for coliform enumeration of chlorine-stressed simulated drinking-water samples were promising (Chapter 3) and should be in concordance with new definitions based on enzyme

activity, in contrast with more traditional methodologies based on lactose fermentation (Anon, 1994; Anon, 2000). The method should prove to be highly cost-effective since only one filter is used for incubation at 37°C only, and the amount of substrate required is extremely small. As previously mentioned, the incorporation of an enzyme substrate into a medium allows *in-situ* detection of the enzyme, simplifying any further confirmatory testing (Sartory and Watkins, 1999). Furthermore, the membrane filtration method employed is one which is well known and no change to current techniques would be required. This is a particular advantage as there would be no requirement to purchase new equipment or to retrain technicians, both of which are important considerations when attempting to adopt new methodologies within a laboratory.

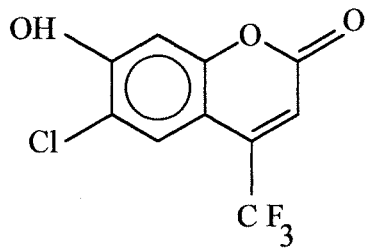
The second aspect of this research concentrated on the development of a fluorogenic coliform assay system which would provide rapid, quantitative results using a MPN-based assay. Preliminary studies were very encouraging: levels of fluorescence generated upon hydrolysis of the novel ß-galactosidase substrates, in particular EHC-GAL, were greatly increased compared to those of the commercially available alternative, 4-MU-GAL. The novel substrates were also, in several cases, less inhibitory to bacterial growth than 4-MU-GAL, although no inhibitory effect on *E. coli* growth by 4-MU-GAL has been reported by other authors (Moberg, 1985). However when 18 h kinetic assays were carried out, problems were encountered with the novel substrate which were most likely to be due to the increased sensitivity and reduced toxicity of EHC-GAL. More extensive studies are required to establish the benefits of such a substrate. It is true that a 'positive' sample can be observed within 4 h when assaying heavily contaminated waters containing coliforms. However, a quantitative

result was generally not available until 18 h. Therefore this remains an overnight assay and confirmatory tests are still required as oxidase-positive organisms, most likely to be *Aeromonas* spp., were isolated in high frequencies. Some studies have suggested that *Aeromonas* strains can be a significant health concern because they are opportunistic pathogens capable of causing a number of serious diseases (Khardori and Fainstein, 1988). Moyer *et al.* (1992) carried out studies which show that *Aeromonas* spp. isolated from water may be a cause of diarrhoeal disease. Most *Aeromonas* spp. are ß-galactosidase positive (Sakazaki and Balows, 1981) and will be detected in any sensitive assay involving a fluorogenic ß-galactoside.

The availability of sophisticated computer software and optical equipment allows rapid and automated screening and image analysis of microcolonies on membrane filters (Sartory and Watkins, 1999). This increase in detection sensitivity reduces the culture period to 4 - 6 h for microcolonies grown on membrane filters placed on a pad soaked with a growth medium incorporating a fluorogenic substrate. Genera Technologies Limited (Cambridgeshire, UK) funded some initial research into the development of a rapid broth-based membrane filtration system. This system required the incorporation of both a fluorogenic galactoside and a fluorogenic glucuronide, potentially allowing simultaneous detection of total coliforms and *E. coli* within 5 hours by the use of automated microscopy to detect fluorescent micro-colonies on membrane filters. In order for this system to work, the respective fluorogens will need to have significantly different excitation and emission wavelengths. This difference in emission wavelengths will mean that the microcolonies will be observed as differently coloured fluorescent colonies, allowing them to be distinguished as either coliform or *E. coli*.

Preliminary studies, using the same methodologies described in Chapter 4, have looked at the potential of two fluorogenic coumarin derivatives for inclusion in a microcolony membrane filtration system. One of these is the novel coumarin core molecule, 6-chloro-7-hydroxy-4-trifluoromethylcoumarin, synthesised by Dr A. L. James, which was thought to exhibit a fluorescence spectrum that was shifted significantly from those of other coumarin derivatives. The structure of this core molecule is shown in Figure 6.1.

**Figure 6.1: Structure of 6-chloro-7-hydroxy-4-trifluoromethylcoumarin**




The fluorescence properties of this core molecule were investigated and a ß-galactosidase substrate synthesised. This substrate was proposed for use with a ß-glucuronidase substrate already described in Chapter 4, namely 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-glucuronide. Figures 6.2a and b illustrate the shifted fluorescence spectra of the two core molecules indicting how suitable they are for inclusion in a dual substrate system. The core molecule of the novel galactoside, 6-chloro-7-hydroxy-4-trifluoromethylcoumarin, has an excitation peak around 400 nm and an emission peak at approximately 500 nm. As illustrated, the excitation peak of 6-chloro-7-hydroxy-4-methylcoumarin is around 375 nm with an emission peak near 450 nm. This combination of fluorescence wavelengths has the added advantage of a possible common excitation wavelength at approximately 380 nm. The fluorescence emission

generated by hydrolysis of the galactoside substrate could be read at a wavelength as high as 500 - 520 nm, where coliform microcolonies would fluoresce green, with the emission of the glucuronide fluorescence at 445 nm so that *E. coli* microcolonies would fluoresce blue. It is important to ensure that the emission of one particular fluorophore is not at a wavelength where the other fluorophore has its excitation peak, this is because any fluoroscence emitted would be absorbed by the latter.

**Figure 6.2a: Excitation spectra of two novel coumarin derivatives at pH 7.4 (0.5 mmol $l^{-1}$)**

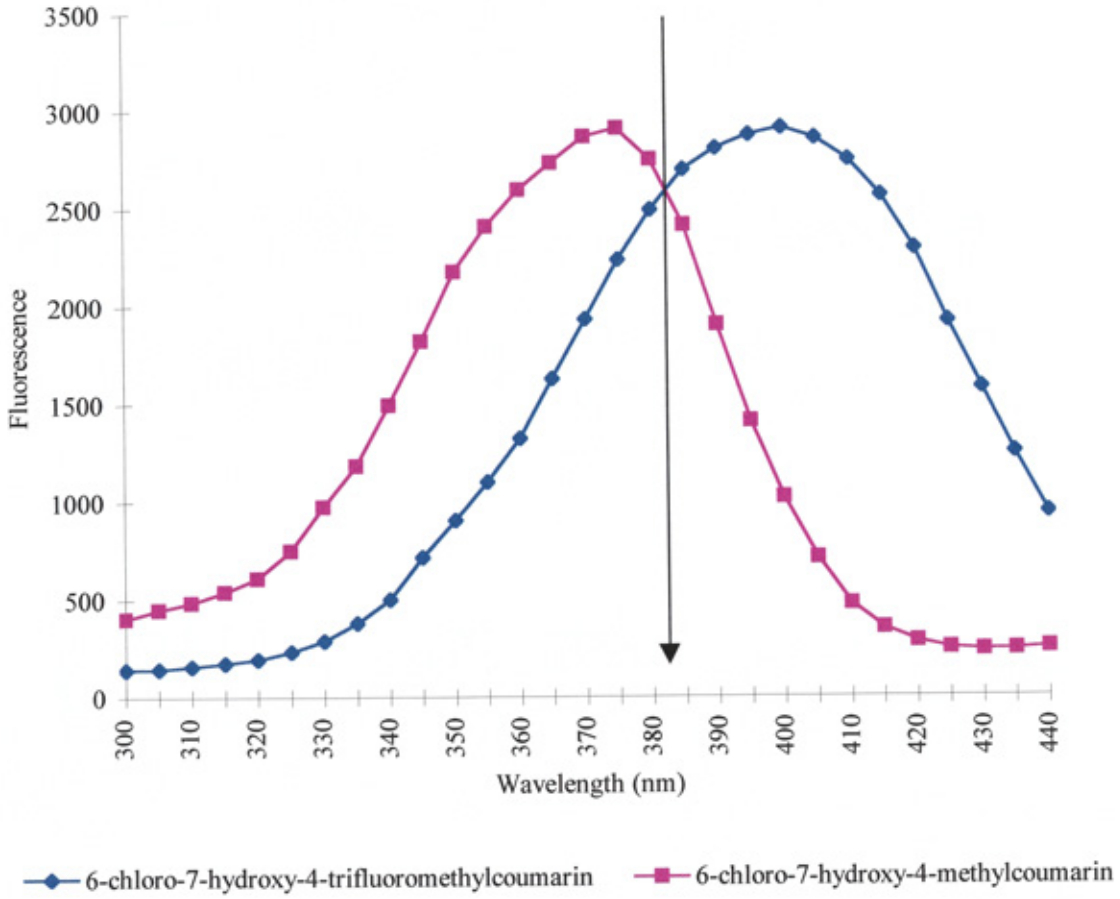
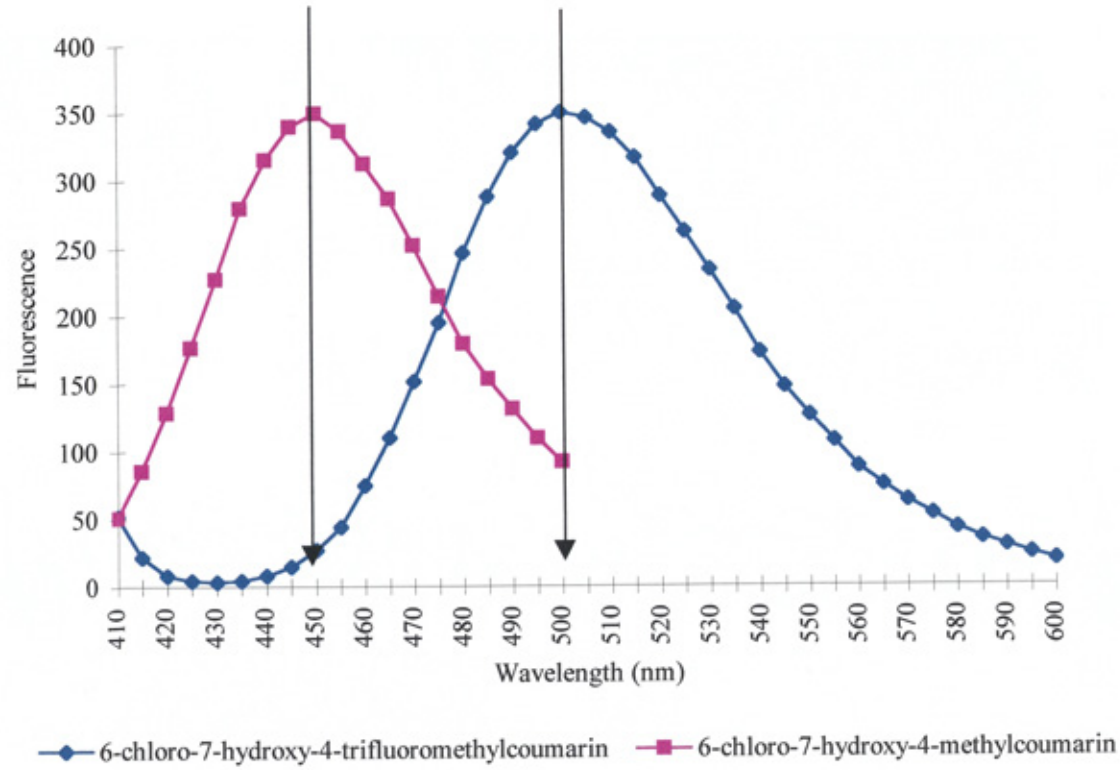



**Figure 6.2b: Emission spectra of two novel coumarin derivatives at pH 7.4 (0.5 mmol $l^{-1}$).**



This dual substrate system is being developed and is proving to work well in preliminary experiments. Figures 6.3 and 6.4 show the results of some initial studies with 6-chloro-7-hydroxy-4-trifluoromethylcoumarin-ß-D-galactoside and 4-trifluoromethylcoumarin-ß-D-galactoside, the latter of which is the commercially available derivative of the core molecule. Toxicity studies to see if these substrates had any potential inhibitory effect on bacterial growth with an *E. coli* strain (FRHECO2) have shown the substrate to be comparable to standard MLSB (Figure 6.3). Furthermore, the fluorescence generated within 6 h (360 min) is encouraging (Figure 6.4). This assay now requires extensive evaluation with a number of coliforms and water samples by Genera Technologies Ltd.

Van Poucke and Nelis (2000) described a two-step procedure for the fluorescent-labelling of microcolonies on a membrane filter using 4-trifluoromethylcoumarin-ß-D-glycosides. The procedure involved a separation of the bacterial propagation and target enzyme induction, plus the use of improved fluorogenic substrates. The authors detected microcolonies of pure cultures within 5.5 - 6.5 h but suggested that waterborne coliforms, including *E. coli*, may take longer to form microcolonies. The fluorescence generated by 6-chloro-4-trifluoromethylcoumarin-ß-D-galactoside, the substrate evaluated for Genera Technologies Limited, was twice that of 4-trifluoromethylcoumarin-ß-D-glycosides, suggesting that detection of microcolonies could potentially be enhanced with the inclusion of this novel substrate.

**Figure 6.3: Growth of *Escherichia coli* (FRHECO2) in the presence of two novel ß-galactosidase substrates.**

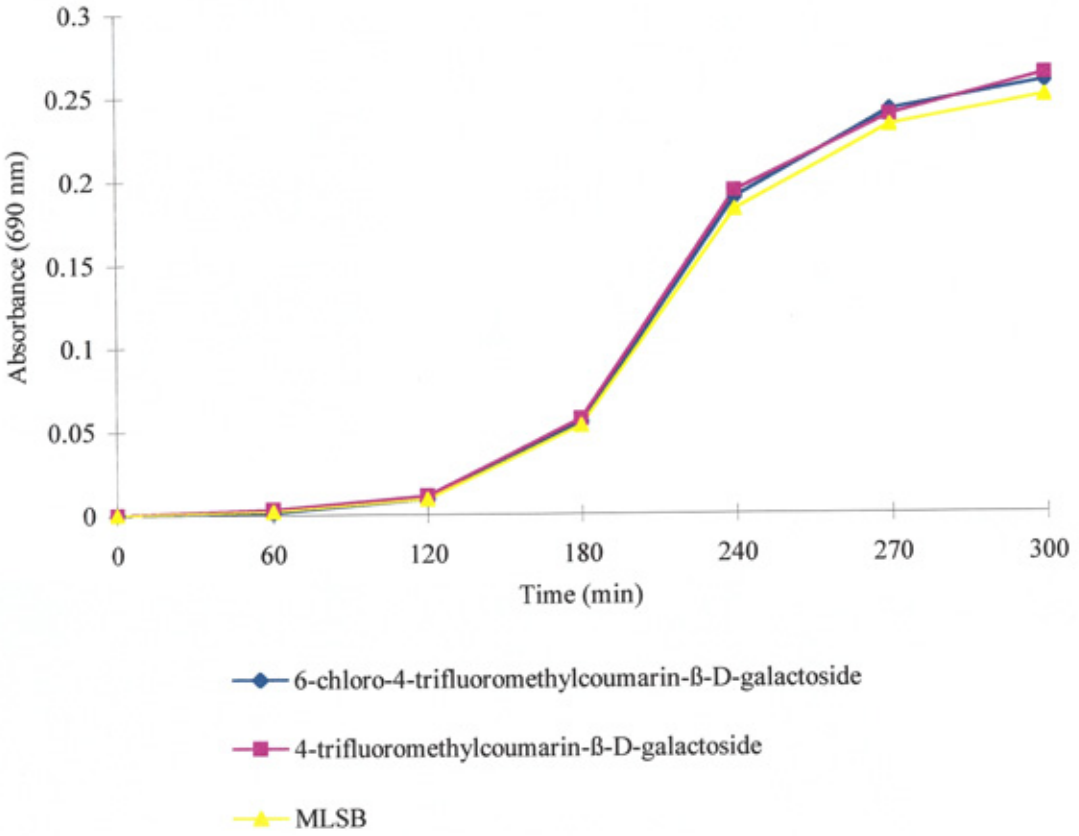
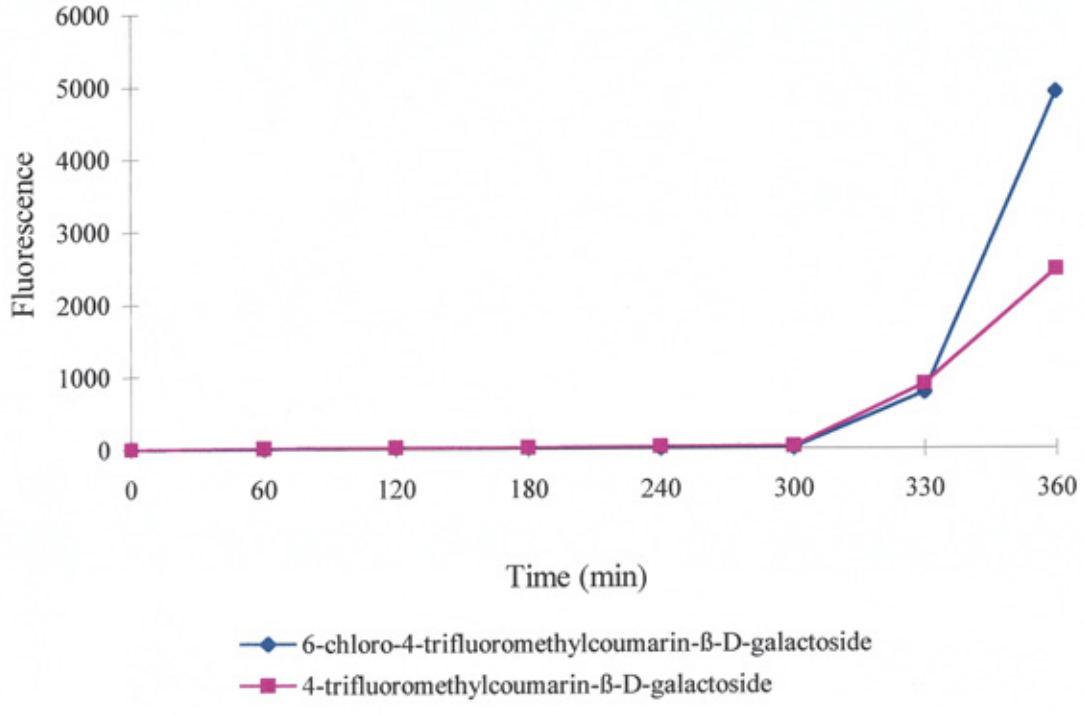



**Figure 6.4: Fluorescence generated by *Escherichia coli* (FRHECO2) when grown in the presence of two novel ß-galactosidase substrates.**



The coumarin core molecules evaluated in this research have great potential for other cellular glycosidase enzymes as well as ß-galactosidases and ß-glucuronidases. Virtually all mammals carry enterococci in the colon, namely *E. faecium* and *E. faecalis*, at concentrations of approximately $10^6$ - $10^7$ g $l^{-1}$ (Edberg *et al.*, 2000). The usefulness of ß-glucosidase for bacterial differentiation of enterococci has been demonstrated in the literature (Bascomb, 1987; Kaufhold *et al.*, 1989). Manafi *et al.* (1991) suggested that the use of 4-MU-ß-D-glucoside could be useful in distinguishing faecal streptococci in a rapid fluorogenic assay. Once again, there is potential for the core molecules evaluated in this study to generate higher levels of fluorescence than substrates based on 4-MU if incorporated into an assay system for enterococci.

The chromogenic and fluorogenic substrates described here have been evaluated and have shown their potential as powerful tools in diagnostic microbiology, utilising specific enzymatic activities of coliforms, either in addition to or in place of standard methodologies. In some cases the substrates have shown increased sensitivity and enhanced recoveries of indicator organisms which can in turn, present novel problems (Chapter 5). Furthermore, with the availability of computer software and optical equipment the potential exists for rapid, automated screening, significantly reducing the time taken to achieve either a positive and/or a quantitative result.

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# APPENDICES

| Appendix 2-1: Evaluation of chromogenic substrates for enumeration of coliforms. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ALIZ-GAL (+ $Fe^{3+}$) | | ALIZ-GAL (+ $Al^{3+}$) | | CHE-GAL (+ $Fe^{3+}$) | | PNB-GAL | |
| | total colonies | number positive | total colonies | number positive | total colonies | number positive | total colonies | number positive |
| *E. coli* (NCTC 10418) | 78 | 78 | 25 | 25 | 119 | 119 | 62 | 62 |
| | 87 | 87 | 38 | 38 | 136 | 136 | 106 | 106 |
| *E. coli* (FRHECO1) | 74 | 74 | 65 | 65 | 56 | 56 | 54 | 54 |
| | 68 | 68 | 81 | 81 | 61 | 61 | 59 | 59 |
| *E. coli* (FRHECO2) | 124 | 124 | 99 | 99 | 98 | 98 | 89 | 89 |
| | 97 | 97 | 86 | 86 | 102 | 102 | 90 | 90 |
| *E. coli* (FRHECO3) | 180 | 180 | 181 | 181 | 163 | 163 | 126 | 126 |
| | 165 | 165 | 176 | 176 | 150 | 150 | 139 | 139 |
| *E. cloacae* (NCTC 11936) | 172 | 172 | 151 | 151 | 160 | 160 | 182 | 182 |
| | 157 | 157 | 164 | 164 | 169 | 169 | 161 | 161 |
| *E. cloacae* (FRHECL7) | 158 | 158 | 144 | 144 | 159 | 159 | 157 | 157 |
| | 150 | 150 | 168 | 168 | 140 | 140 | 156 | 156 |
| *E. cloacae* (FRHECL8) | 154 | 154 | 131 | 131 | 158 | 158 | 103 | 103 |
| | 131 | 131 | 136 | 136 | 140 | 140 | 111 | 111 |
| *E. cloacae* (FRHECL9) | 201 | 201 | 148 | 148 | 170 | 170 | 181 | 181 |
| | 178 | 178 | 140 | 140 | 156 | 156 | 180 | 180 |
| *H. alvei* (FRHHAL2) | 143 | 111 | 133 | 101 | 159 | 113 | 118 | 118 |
| | 136 | 99 | 146 | 110 | 136 | 99 | 128 | 128 |
| *H. alvei* (FRHHAL3) | 504 | 0 | 256 | 0 | 226 | 0 | 196 | 0 |
| | 198 | 0 | 274 | 0 | 170 | 0 | 204 | 0 |
| *H. alvei* (FRHHAL4) | 180 | 180 | 117 | 0 | 135 | 0 | 144 | 0 |
| | 207 | 207 | 126 | 0 | 99 | 0 | 189 | 0 |
| *H. alvei* (FRHHAL5) | 83 | 83 | 73 | 0 | 88 | 0 | 72 | 0 |
| | 75 | 75 | 105 | 0 | 69 | 0 | 84 | 0 |
| *C. freundii* (NCTC 9750) | 109 | 109 | 106 | 106 | 122 | 122 | 106 | 106 |
| | 108 | 108 | 111 | 111 | 111 | 111 | 107 | 107 |
| *C. freundii* (FRHCFR1) | 176 | 176 | 150 | 150 | 147 | 147 | 152 | 152 |
| | 151 | 151 | 140 | 140 | 147 | 147 | 141 | 141 |
| *C. freundii* (FRHCFR2) | 86 | 86 | 81 | 81 | 99 | 99 | 86 | 86 |
| | 87 | 87 | 79 | 79 | 84 | 84 | 84 | 84 |
| *C. freundii* (FRHCFR3) | 94 | 94 | 106 | 106 | 98 | 98 | 88 | 88 |
| | 96 | 96 | 84 | 84 | 83 | 83 | 92 | 92 |
| *K. pneumoniae* (NCTC 10896) | NG | NG | NG | NG | 14 | 14 | NG | NG |
| | NG | NG | 1 | 1 | 59 | 59 | NG | NG |
| *K. pneumoniae* (FRHKPN9) | 121 | 121 | 138 | 138 | 116 | 116 | 114 | 114 |
| | 129 | 129 | 118 | 118 | 126 | 126 | 116 | 116 |
| *K. pneumoniae* (FRHKPN10) | 64 | 64 | 70 | 70 | 62 | 62 | 64 | 64 |
| | 57 | 57 | 60 | 60 | 70 | 70 | 58 | 58 |
| *K. pneumoniae* (FRHKPN11) | 65 | 65 | 76 | 76 | 63 | 63 | 61 | 61 |
| | 66 | 66 | 75 | 75 | 72 | 72 | 77 | 77 |
| *S. marcescens* (NCTC 10211) | 312 | 312 | 288 | 288 | 316 | 316 | 328 | 328 |
| | 268 | 268 | 246 | 246 | 356 | 356 | 320 | 320 |
| *Serratia* spp. (FRHSEX1) | 216 | 216 | 206 | 206 | 252 | 252 | 256 | 256 |
| | 268 | 268 | 236 | 236 | 252 | 252 | 328 | 328 |
| *Serratia* spp. (FRHSEX2) | 285 | 285 | 301 | 301 | 264 | 264 | 279 | 279 |
| | 274 | 274 | 288 | 288 | 277 | 277 | 299 | 299 |
| *Serratia* spp. (FRHSEX3) | 298 | 298 | 306 | 306 | 284 | 284 | 320 | 320 |
| | 324 | 324 | 328 | 328 | 304 | 304 | 300 | 300 |
| *Y. enterocolitica* (NCTC 11176) | 102 | 0 | 111 | 0 | 113 | 0 | 122 | 0 |
| | 92 | 0 | 103 | 0 | 110 | 0 | 118 | 0 |
| *Y. enterocolitica* (NCTC 10938) | 88 | 0 | 71 | 0 | 86 | 0 | 67 | 0 |
| | 74 | 0 | 87 | 0 | 79 | 0 | 76 | 0 |
| *Y. enterocolitica* (NCTC 10463) | 65 | 0 | 71 | 0 | 76 | 0 | 80 | 0 |
| | 85 | 0 | 74 | 0 | 89 | 0 | 86 | 0 |
| *Y. enterocolitica* (NCTC 10461) | 100 | 0 | 88 | 0 | 116 | 0 | 105 | 0 |
| | 124 | 0 | 94 | 0 | 113 | 0 | 109 | 0 |

| Appendix 2-1 (Cont'd..): Evaluation of chromogenic substrates for enumeration of coliforms. | | | | | | | |
|---|---|---|---|---|---|---|---|
| | X-GAL | | DHN-GAL (+ $Fe^{3+}$) | | m-ENDO | | columbia |
| | total colonies | number positive | total colonies | number positive | total colonies | number positive | |
| *E. coli* (NCTC 10418) | 17 | 17 | 121 | 121 | NG | NG | 146 |
| | 16 | 16 | 118 | 118 | NG | NG | 164 |
| *E. coli* (FRHECO1) | 48 | 48 | 50 | 50 | 38 | 38 | 63 |
| | 55 | 55 | 20 | 20 | 56 | 56 | 65 |
| *E. coli* (FRHECO2) | 93 | 93 | 106 | 106 | 100 | 0 | 95 |
| | 99 | 99 | 30 | 30 | 83 | 0 | 74 |
| *E. coli* (FRHECO3) | 139 | 139 | 162 | 162 | 149 | 149 | 136 |
| | 144 | 144 | 150 | 150 | 163 | 163 | 149 |
| *E. cloacae* (NCTC 11936) | 166 | 166 | 149 | 149 | 136 | 136 | 201 |
| | 179 | 179 | 169 | 169 | 140 | 140 | 195 |
| *E. cloacae* (FRHECL7) | 133 | 133 | 127 | 127 | 161 | 161 | 150 |
| | 138 | 138 | 149 | 149 | 129 | 129 | 143 |
| *E. cloacae* (FRHECL8) | 164 | 164 | 160 | 160 | 150 | 0 | 140 |
| | 151 | 151 | 41 | 41 | 172 | 0 | 135 |
| *E. cloacae* (FRHECL9) | 199 | 199 | 180 | 180 | 180 | 180 | 205 |
| | 162 | 162 | 141 | 141 | 199 | 199 | 191 |
| *H. alvei* (FRHHAL2) | 93 | 93 | 146 | 146 | 108 | 108 | 139 |
| | 101 | 101 | 108 | 108 | 71 | 71 | 149 |
| *H. alvei* (FRHHAL3) | 202 | 0 | 206 | 0 | 210 | 0 | 210 |
| | 185 | 0 | 195 | 0 | 201 | 0 | 200 |
| *H. alvei* (FRHHAL4) | 179 | 0 | 135 | 0 | 171 | 0 | 126 |
| | 171 | 0 | 117 | 0 | 135 | 0 | 189 |
| *H. alvei* (FRHHAL5) | 79 | 0 | 56 | 0 | 75 | 0 | 90 |
| | 71 | 0 | 78 | 0 | 68 | 0 | 91 |
| *C. freundii* (NCTC 9750) | 104 | 104 | 91 | 91 | 96 | 0 | 92 |
| | 102 | 102 | 90 | 90 | 84 | 0 | 104 |
| *C. freundii* (FRHCFR1) | 138 | 138 | 170 | 170 | 162 | 162 | 144 |
| | 118 | 118 | 146 | 146 | 150 | 150 | 108 |
| *C. freundii* (FRHCFR2) | 75 | 75 | 63 | 63 | 79 | 79 | 69 |
| | 83 | 83 | 88 | 88 | 89 | 89 | 74 |
| *C. freundii* (FRHCFR3) | 106 | 106 | 95 | 95 | 101 | 101 | 85 |
| | 88 | 88 | 83 | 83 | 88 | 88 | 72 |
| *K. pneumoniae* (NCTC 10896) | 93 | 93 | NG | NG | 1 | 1 | 104 |
| | 81 | 81 | NG | NG | NG | NG | 96 |
| *K. pneumoniae* (FRHKPN9) | 123 | 123 | 119 | 119 | 121 | 121 | 125 |
| | 105 | 105 | 126 | 126 | 105 | 105 | 109 |
| *K. pneumoniae* (FRHKPN10) | 63 | 63 | 67 | 67 | 51 | 51 | 75 |
| | 65 | 65 | 73 | 73 | 63 | 63 | 60 |
| *K. pneumoniae* (FRHKPN11) | 72 | 72 | 68 | 68 | 73 | 73 | 68 |
| | 81 | 81 | 68 | 68 | 61 | 61 | 64 |
| *S. marcescens* (NCTC 10211) | 340 | 340 | 396 | 396 | 336 | 0 | 302 |
| | 296 | 296 | 352 | 352 | 368 | 0 | 332 |
| *Serratia* spp. (FRHSEX1) | 248 | 248 | 316 | 316 | 204 | 0 | 300 |
| | 256 | 256 | 288 | 288 | 188 | 0 | 288 |
| *Serratia* spp. (FRHSEX2) | 242 | 242 | 301 | 301 | 222 | 0 | 245 |
| | 289 | 289 | 259 | 259 | 241 | 0 | 265 |
| *Serratia* spp. (FRHSEX3) | 310 | 310 | 312 | 312 | 280 | 0 | 280 |
| | 312 | 312 | 298 | 298 | 308 | 0 | 298 |
| *Y. enterocolitica* (NCTC 11176) | 115 | 0 | 89 | 0 | 126 | 0 | 135 |
| | 124 | 0 | 92 | 0 | 111 | 0 | 120 |
| *Y. enterocolitica* (NCTC 10938) | 77 | 0 | 77 | 0 | 63 | 0 | 65 |
| | 74 | 0 | 78 | 0 | 87 | 0 | 73 |
| *Y. enterocolitica* (NCTC 10463) | 70 | 0 | 90 | 0 | 75 | 0 | 89 |
| | 82 | 0 | 91 | 0 | 79 | 0 | 85 |
| *Y. enterocolitica* (NCTC 10461) | 119 | 0 | 113 | 0 | 89 | 0 | 113 |
| | 96 | 0 | 109 | 0 | 90 | 0 | 110 |

| **Appendix 2.2: Evaluation of chromogenic substrates for enumeration of coliforms:** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| **Statistical analysis of averaged total colony counts: t-test when compared with Columbia agar.** | | | | | | | | |
| | ALIZ-GAL (+ $Fe^{3+}$) | ALIZ-GAL (+ $Al^{3+}$) | CHE-GAL (+ $Fe^{3+}$) | PNB-GAL | X-GAL | DHN-GAL (+ $Fe^{3+}$) | m-ENDO | Columbia |
| Mean | 144.2857 | 133.3214 | 138.75 | 136.2679 | 134.48 | 134.3214 | 126.536 | 142.786 |
| Variance | 7184.101 | 6050.43 | 5355.639 | 6684.009 | 5803.1 | 7580.763 | 6375.83 | 5379.53 |
| Observations | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 |
| Pearson Correlation | 0.865259 | 0.852831 | 0.953882 | 0.94603 | 0.9171 | 0.946021 | 0.85106 | |
| Hypothesized Mean Difference | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| df | 27 | 27 | 27 | 27 | 27 | 27 | 27 | |
| t Stat | -0.18679 | 1.215003 | 0.959729 | 1.287136 | 1.4377 | 1.511789 | 2.03416 | |
| P(T<=t) one-tail | 0.426609 | 0.117442 | 0.172854 | 0.104486 | 0.081 | 0.071102 | 0.02593 | |
| t Critical one-tail | 1.703288 | 1.703288 | 1.703288 | 1.703288 | 1.7033 | 1.703288 | 1.70329 | |
| P(T<=t) two-tail | 0.853218 | 0.234884 | 0.345708 | 0.208972 | 0.162 | 0.142205 | 0.05187 | |
| t Critical two-tail | 2.051829 | 2.051829 | 2.051829 | 2.051829 | 2.0518 | 2.051829 | 2.05183 | |
| | | | | | | | | |
| **Statistical analysis of averaged ß-galactosidase-positive colony counts: t-test when compared with m-ENDO agar.** | | | | | | | | |
| | ALIZ-GAL (+ $Fe^{3+}$) | ALIZ-GAL (+ $Al^{3+}$) | CHE-GAL (+ $Fe^{3+}$) | PNB-GAL | X-GAL | DHN-GAL (+ $Fe^{3+}$) | m-ENDO | Columbia |
| Mean | 117.5536 | 102.4464 | 109.4107 | 106.75 | 105.13 | 107.0714 | 47.7321 | |
| Variance | 8321.469 | 8651.247 | 8927.52 | 9981.454 | 8986.5 | 10873.72 | 3984.06 | |
| Observations | 28 | 28 | 28 | 28 | 28 | 28 | 28 | |
| Pearson Correlation | 0.174877 | 0.23334 | 0.166545 | 0.186891 | 0.1718 | 0.159822 | | |
| Hypothesized Mean Difference | 0 | 0 | 0 | 0 | 0 | 0 | | |
| df | 27 | 27 | 27 | 27 | 27 | 27 | | |
| t Stat | -3.64187 | -2.910456 | -3.12249 | -2.89852 | -2.907 | -2.78035 | | |
| P(T<=t) one-tail | 0.000566 | 0.003574 | 0.002123 | 0.003679 | 0.0036 | 0.004886 | | |
| t Critical one-tail | 1.703288 | 1.703288 | 1.703288 | 1.703288 | 1.7033 | 1.703288 | | |
| P(T<=t) two-tail | 0.001132 | 0.007148 | 0.004246 | 0.007357 | 0.0072 | 0.009772 | | |
| t Critical two-tail | 2.051829 | 2.051829 | 2.051829 | 2.051829 | 2.0518 | 2.051829 | | |

| Appendix 2-3: Evaluation of chromogenic substrates for enumeration of UV-A damaged coliforms: Total counts. | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ALIZ-GAL (+ $Fe^{3+}$) | | ALIZ-GAL (+ $Al^{3+}$) | | CHE-GAL (+ $Fe^{3+}$) | | PNB-GAL | | X-GAL | | DHN-GAL (+ $Fe^{3+}$) | | m-ENDO | | Columbia |
| | total colonies | number positive | total colonies | number positive | total colonies | number positive | total colonies | number positive | total colonies | number positive | total colonies | number positive | total colonies | number positive | total colonies |
| *E. coli* (FRHECO2) | 135 | 135 | 128 | 128 | 136 | 136 | 165 | 165 | 178 | 178 | 132 | 132 | 123 | 123 | 162 |
| | 123 | 123 | 141 | 141 | 123 | 123 | 126 | 126 | 146 | 146 | 137 | 137 | 127 | 127 | 156 |
| *E. coli* (FRHECO3) | 51 | 51 | 59 | 59 | 59 | 59 | 60 | 60 | 59 | 59 | 70 | 70 | 52 | 52 | 191 |
| | 44 | 44 | 51 | 51 | 45 | 45 | 61 | 61 | 45 | 45 | 66 | 66 | 63 | 63 | 170 |
| *K. pneumoniae* (FRHKPN9) | 148 | 148 | 160 | 160 | 172 | 172 | 152 | 152 | 144 | 144 | 180 | 180 | 124 | 124 | 174 |
| | 156 | 156 | 172 | 172 | 192 | 192 | 175 | 175 | 148 | 148 | 164 | 164 | 148 | 148 | 178 |
| *K. pneumoniae* (FRHKPN10) | 208 | 208 | 190 | 190 | 200 | 200 | 216 | 216 | 152 | 152 | 180 | 180 | 192 | 192 | 152 |
| | 168 | 168 | 156 | 156 | 168 | 168 | 164 | 164 | 160 | 160 | 164 | 164 | 138 | 138 | 128 |
| *C. freundii* (FRHCFR2) | 144 | 144 | 184 | 184 | 180 | 180 | 168 | 168 | 152 | 152 | 188 | 188 | 132 | 132 | 208 |
| | 144 | 144 | 140 | 140 | 180 | 180 | 200 | 200 | 148 | 148 | 156 | 156 | 164 | 164 | 208 |
| *C. freundii* (FRHCFR3) | 136 | 136 | 112 | 112 | 136 | 136 | 108 | 108 | 132 | 132 | 144 | 144 | 112 | 112 | 156 |
| | 132 | 132 | 120 | 120 | 142 | 142 | 112 | 112 | 142 | 142 | 142 | 142 | 120 | 120 | 164 |

## Appendix 3.1: Free and total chlorine analysis in potable waters

Forms of chlorine are determined as two separate types; free available chlorine defined as that residual chlorine existing in water as hypochlorous and hypochlorite ion, or, combined available chlorine defined as that residual chlorine existing in water in chemical combination with ammonia or organic nitrogen compounds. The sum of both chlorine types is known as residual available chlorine.

The assay described here depends on the formation of a red colour between chlorine and diethyl-p-phenylenediamine (DPD). This was followed by a titration with ferrous ammonium sulphate. Before the assay, a calibration procedure is necessary. A 100 ml aliquot of standard ferrous ammonium sulphate (FAS) solution was pipetted into a conical flask. A 20 ml volume of 10% v/v sulphuric acid, 5 ml of concentrated phosphoric acid and 2 ml of 0.1 % barium diphenylamine indicator was then added. The mixture was titrated with potassium dichromate to a violet end point that persisted for approximately 30 seconds. This volume was denoted 'S' ml. A factor 'F', for the strength of FAS in terms of its chlorine equivalent was calculated from F = S/2.82. If the factor was within 1.00 +/- 0.02 then no correction was necessary on titrations.

**Measuring free available chlorine**

To a conical flask 5 ml of N,N-Diethyl-p-phenylenediamine (DPD) indicator solution was added, followed by 5 ml buffer solution. The conical flask was mixed followed by the addition of a 100 ml sample of test water, this solution was mixed well. The

solution was titrated immediately with FAS solution until the colour was discharged. This titration was 'A' ml.

**Measuring combined available chlorine**

To the solution obtained from measuring 'free available chlorine' after the free chlorine determination, approximately 0.1 g potassium iodide was added and swirled to aid dissolution. A titratration with FAS solution was carried out until the colour persisted for approximately 2 minutes. This titration was 'B' ml.

**Measuring total available chlorine**

To a 250 ml conical flask, 5 ml of DPD solution was added followed by 5 ml of buffer solution and mixing. Approximately 0.1 g of potassium iodide was added and swirled to aid dissolution. This was followed by the addition of a 100 ml sample of test water and mixing. After 2 minutes the solution was titrated with FAS solution. This titration was 'C' ml.

Free available chlorine = A x F mg/l $Cl_2$

Combined available chlorine = B x F mg/l $Cl_2$

Total available chlorine = C x F mg/l $Cl_2$

(Where F is the factor derived from the calibration procedure (normally 1)).

**Appendix 3.2: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media.**

| | MLSB (37°C) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | MLSB (44°C) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | total | yellow | /10 | | | | % | % | total | yellow | /10 | | | | % | % |
| 1 | 53 | 35 | 10 | 10 | 10 | 2 | 100 | 20 | 7 | 6 | 6 | 6 | 6 | 1 | 100 | 17 |
| 2 | 46 | 35 | 9 | 9 | 8 | 3 | 90 | 30 | 8 | 7 | 7 | 7 | 6 | 3 | 100 | 43 |
| 3 | 42 | 32 | 10 | 10 | 10 | 3 | 100 | 30 | 10 | 8 | 8 | 8 | 8 | 6 | 100 | 75 |
| 4 | 33 | 25 | 10 | 10 | 10 | 3 | 100 | 30 | 4 | 4 | 4 | 3 | 3 | 2 | 75 | 50 |
| 5 | 43 | 30 | 10 | 10 | 10 | 2 | 100 | 20 | 2 | 2 | 2 | 2 | 2 | 2 | 100 | 100 |
| 6 | 27 | 20 | 9 | 9 | 9 | 1 | 90 | 10 | 3 | 3 | 3 | 2 | 1 | 0 | 67 | 0 |
| 7 | 23 | 15 | 10 | 10 | 10 | 3 | 100 | 30 | 6 | 5 | 5 | 5 | 5 | 5 | 100 | 100 |
| 8 | 31 | 22 | 10 | 10 | 10 | 6 | 100 | 60 | 10 | 8 | 8 | 8 | 8 | 1 | 100 | 13 |
| 9 | 31 | 23 | 10 | 10 | 9 | 4 | 100 | 40 | 5 | 4 | 4 | 4 | 4 | 1 | 100 | 25 |
| 10 | 42 | 23 | 10 | 10 | 10 | 2 | 100 | 20 | 8 | 7 | 7 | 7 | 7 | 0 | 100 | 0 |
| | | | | | | | | | | | | | | | | |
| | MLSB (37°C) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | MLSB (44°C) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* |
| | total | yellow | /10 | | | | % | % | total | yellow | /10 | | | | % | % |
| 11 | 94 | 34 | 9 | 9 | 9 | 5 | 90 | 50 | 19 | 13 | 10 | 10 | 10 | 4 | 100 | 40 |
| 12 | 87 | 33 | 10 | 10 | 10 | 3 | 100 | 30 | 18 | 16 | 10 | 10 | 10 | 7 | 100 | 70 |
| 13 | 92 | 35 | 10 | 10 | 10 | 7 | 100 | 70 | 17 | 11 | 10 | 10 | 10 | 5 | 100 | 50 |
| 14 | 65 | 23 | 10 | 10 | 10 | 3 | 100 | 30 | 17 | 12 | 10 | 10 | 10 | 4 | 100 | 40 |
| 15 | 61 | 31 | 10 | 10 | 10 | 2 | 100 | 20 | 12 | 10 | 10 | 10 | 10 | 6 | 100 | 60 |
| 16 | 36 | 22 | 10 | 10 | 10 | 5 | 100 | 50 | 11 | 10 | 10 | 10 | 10 | 6 | 100 | 60 |
| 17 | 63 | 29 | 10 | 10 | 10 | 2 | 100 | 20 | 9 | 4 | 4 | 4 | 4 | 2 | 100 | 50 |
| 18 | 46 | 20 | 10 | 10 | 10 | 5 | 100 | 50 | 8 | 4 | 4 | 4 | 4 | 2 | 100 | 50 |
| 19 | 47 | 16 | 10 | 10 | 10 | 5 | 100 | 50 | 7 | 6 | 6 | 6 | 6 | 2 | 100 | 33 |
| 20 | 52 | 25 | 10 | 10 | 10 | 6 | 100 | 60 | 6 | 4 | 4 | 4 | 4 | 2 | 100 | 50 |

**Appendix 3.2: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media.**

| | MLSB (37°C) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | MLSB (44°C) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | total | yellow | /10 | | | | % | % | total | yellow | | | | | % | % |
| 21 | 12 | 12 | 10 | 10 | 10 | 5 | 100 | 50 | 12 | 11 | 10 | 10 | 10 | 5 | 100 | 50 |
| 22 | 13 | 10 | 10 | 10 | 10 | 5 | 100 | 50 | 13 | 11 | 10 | 10 | 10 | 4 | 100 | 40 |
| 23 | 14 | 12 | 10 | 10 | 10 | 3 | 100 | 30 | 3 | 3 | 3 | 3 | 3 | 1 | 100 | 33 |
| 24 | 17 | 14 | 10 | 10 | 10 | 1 | 100 | 10 | 6 | 6 | 6 | 6 | 6 | 2 | 100 | 33 |
| 25 | 20 | 17 | 10 | 10 | 10 | 4 | 100 | 40 | 3 | 2 | 2 | 2 | 2 | 0 | 100 | 0 |
| 26 | 18 | 12 | 10 | 10 | 10 | 4 | 100 | 40 | 2 | 2 | 2 | 2 | 2 | 1 | 100 | 50 |
| 27 | 70 | 54 | 10 | 10 | 10 | 4 | 100 | 40 | 15 | 14 | 10 | 10 | 10 | 4 | 100 | 40 |
| 28 | 56 | 49 | 10 | 10 | 10 | 2 | 100 | 20 | 14 | 14 | 10 | 10 | 10 | 6 | 100 | 60 |
| 29 | 56 | 46 | 10 | 10 | 10 | 5 | 100 | 50 | 12 | 12 | 10 | 10 | 10 | 8 | 100 | 80 |
| 30 | 50 | 40 | 10 | 10 | 10 | 3 | 100 | 30 | 10 | 7 | 7 | 7 | 7 | 3 | 100 | 43 |
| | | | | | | | | | | | | | | | | |
| | MLSB (37°C) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | MLSB (44°C) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* |
| | total | yellow | /10 | | | | % | % | total | yellow | | | | | % | % |
| 31 | 30 | 20 | 10 | 10 | 10 | 0 | 100 | 0 | 24 | 21 | 10 | 9 | 9 | 0 | 90 | 0 |
| 32 | 28 | 19 | 10 | 9 | 9 | 2 | 90 | 20 | 34 | 27 | 10 | 8 | 8 | 1 | 80 | 10 |
| 33 | 41 | 24 | 10 | 8 | 8 | 2 | 80 | 20 | 18 | 13 | 10 | 8 | 8 | 2 | 80 | 20 |
| 34 | 21 | 11 | 9 | 9 | 9 | 1 | 90 | 10 | 15 | 12 | 7 | 6 | 6 | 0 | 60 | 0 |
| 35 | 28 | 25 | 10 | 10 | 9 | 1 | 100 | 10 | 19 | 13 | 10 | 10 | 10 | 2 | 100 | 20 |
| 36 | 38 | 28 | 10 | 10 | 10 | 1 | 100 | 10 | 32 | 24 | 10 | 10 | 10 | 1 | 100 | 10 |
| 37 | 47 | 38 | 10 | 10 | 10 | 1 | 100 | 10 | 27 | 21 | 10 | 9 | 9 | 1 | 90 | 10 |
| 38 | 46 | 25 | 10 | 10 | 10 | 5 | 100 | 50 | 25 | 15 | 10 | 10 | 10 | 1 | 100 | 10 |
| 39 | 45 | 34 | 10 | 10 | 10 | 3 | 100 | 30 | 28 | 24 | 9 | 7 | 7 | 3 | 70 | 30 |
| 40 | 29 | 22 | 10 | 10 | 10 | 1 | 100 | 10 | 7 | 7 | 5 | 5 | 5 | 2 | 71 | 29 |

| Appendix 3.2: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media. | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MLSB (37°C) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | MLSB (44°C) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* |
| | total | yellow | /10 | | | | % | % | total | yellow | | | | | % | % |
| 41 | 79 | 16 | 10 | 9 | 9 | 9 | 90 | 90 | 2 | 2 | 2 | 2 | 2 | 2 | 100 | 100 |
| 42 | 23 | 9 | 9 | 4 | 4 | 4 | 44 | 44 | 3 | 1 | 1 | 1 | 1 | 1 | 100 | 100 |
| 43 | 22 | 8 | 7 | 6 | 5 | 5 | 75 | 63 | 2 | 1 | 1 | 1 | 1 | 1 | 100 | 100 |
| 44 | 55 | 7 | 6 | 6 | 6 | 6 | 86 | 86 | 3 | 1 | 1 | 1 | 1 | 0 | 100 | 0 |
| 45 | 41 | 18 | 10 | 8 | 8 | 8 | 80 | 80 | 13 | 1 | 1 | 1 | 1 | 1 | 100 | 100 |
| 46 | 38 | 15 | 9 | 9 | 9 | 9 | 90 | 90 | 31 | 6 | 6 | 6 | 6 | 6 | 100 | 100 |
| 47 | 100 | 75 | 6 | 2 | 2 | 2 | 20 | 20 | 19 | 3 | 3 | 3 | 3 | 2 | 100 | 67 |
| 48 | 200 | 110 | 9 | 2 | 2 | 2 | 20 | 20 | 13 | 2 | 2 | 2 | 2 | 2 | 100 | 100 |
| 49 | 150 | 90 | 10 | 1 | 1 | 1 | 10 | 10 | 15 | 2 | 2 | 2 | 2 | 2 | 100 | 100 |
| 50 | 100 | 50 | 7 | 2 | 2 | 2 | 20 | 20 | 5 | 2 | 2 | 2 | 2 | 2 | 100 | 100 |

**Appendix 3.2 Cont'd..: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media.**

| | mMLSB1 (ß-gal) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of ß-gal-positive/LPW(37°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | blue | /10 | | | | % | % | |
| 1 | 43 | 33 | 10 | 8 | 7 | 0 | 80 | 0 | *E.cloacae* and *E.amnigenus* |
| 2 | 38 | 15 | 10 | 9 | 9 | 2 | 90 | 20 | *E.amnigenus* |
| 3 | 41 | 21 | 10 | 10 | 7 | 2 | 100 | 20 | |
| 4 | 44 | 20 | 10 | 10 | 9 | 0 | 100 | 0 | |
| 5 | 30 | 13 | 10 | 9 | 8 | 3 | 90 | 30 | *E.amnigenus* |
| 6 | 29 | 16 | 10 | 9 | 8 | 0 | 90 | 0 | *E.amnigenus* |
| 7 | 20 | 12 | 10 | 9 | 9 | 1 | 90 | 10 | *Kluyvera spp.* |
| 8 | 25 | 13 | 9 | 5 | 1 | 0 | 50 | 0 | *E.cloacae, E.amnigenus(2), E.asburiae* |
| 9 | 36 | 24 | 9 | 9 | 9 | 1 | 90 | 10 | |
| 10 | 27 | 14 | 10 | 10 | 8 | 2 | 100 | 20 | |

| | mMLSB1 (ß-gal) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of ß-gal-positive/LPW(37°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | blue | /10 | | | | % | % | |
| 11 | 40 | 36 | 10 | 10 | 10 | 4 | 100 | 40 | |
| 12 | 29 | 23 | 10 | 10 | 9 | 3 | 100 | 30 | |
| 13 | 30 | 27 | 10 | 10 | 10 | 3 | 100 | 30 | |
| 14 | 28 | 21 | 10 | 10 | 10 | 2 | 100 | 20 | |
| 15 | 31 | 26 | 10 | 10 | 10 | 3 | 100 | 30 | |
| 16 | 16 | 13 | 10 | 10 | 10 | 4 | 100 | 40 | |
| 17 | 25 | 14 | 10 | 10 | 8 | 1 | 100 | 10 | |
| 18 | 28 | 20 | 10 | 9 | 9 | 1 | 90 | 10 | *E.cloacae* |
| 19 | 11 | 6 | 6 | 5 | 5 | 2 | 83 | 20 | *E.cloacae* |
| 20 | 21 | 15 | 10 | 10 | 10 | 2 | 100 | 20 | |

**Appendix 3.2 Cont'd..: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media.**

| | mMLSB1 (ß-gal) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of ß-gal-positive/LPW(37°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | blue | /10 | | | | % | % | |
| 21 | 51 | 35 | 10 | 9 | 9 | 2 | 90 | 20 | *E.cloacae* |
| 22 | 61 | 49 | 10 | 10 | 10 | 2 | 100 | 20 | |
| 23 | 17 | 16 | 10 | 8 | 8 | 4 | 80 | 40 | *C.freundii/E.cloacae* |
| 24 | 8 | 7 | 7 | 7 | 7 | 4 | 100 | 57 | |
| 25 | 18 | 17 | 10 | 10 | 10 | 1 | 100 | 10 | |
| 26 | 8 | 6 | 6 | 6 | 6 | 2 | 100 | 33 | |
| 27 | 48 | 36 | 10 | 9 | 9 | 1 | 90 | 10 | *E.cloacae* |
| 28 | 40 | 34 | 10 | 10 | 10 | 2 | 100 | 20 | |
| 29 | 42 | 29 | 10 | 10 | 10 | 0 | 100 | 0 | |
| 30 | 47 | 39 | 10 | 9 | 9 | 2 | 90 | 20 | *E.cloacae* |

| | mMLSB1 (ß-gal) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of ß-gal-positive/LPW(37°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | blue | /10 | | | | % | % | |
| 31 | 40 | 39 | 10 | 10 | 10 | 1 | 100 | 10 | |
| 32 | 23 | 22 | 10 | 10 | 10 | 1 | 100 | 10 | |
| 33 | 13 | 13 | 10 | 10 | 10 | 0 | 100 | 0 | |
| 34 | 29 | 25 | 10 | 10 | 10 | 5 | 100 | 50 | |
| 35 | 14 | 14 | 10 | 10 | 10 | 2 | 100 | 20 | |
| 36 | 31 | 29 | 10 | 10 | 10 | 2 | 100 | 20 | |
| 37 | 33 | 33 | 10 | 10 | 9 | 2 | 100 | 20 | |
| 38 | 40 | 35 | 10 | 10 | 10 | 4 | 100 | 40 | |
| 39 | 25 | 23 | 10 | 10 | 10 | 2 | 100 | 20 | |
| 40 | 31 | 29 | 10 | 9 | 9 | 7 | 90 | 70 | *K.pneumoniae* |

| Appendix 3.2 Cont'd..: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mMLSB1 (ß-gal) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of ß-gal-positive/LPW(37°C)-negative organisms | |
| | total | blue | /10 | | | | % | % | | |
| 41 | 25 | 8 | 8 | 8 | 8 | 8 | 100 | 100 | | |
| 42 | 17 | 4 | 4 | 4 | 4 | 4 | 100 | 100 | | |
| 43 | 17 | 3 | 3 | 3 | 3 | 3 | 100 | 100 | | |
| 44 | 21 | 6 | 6 | 6 | 6 | 6 | 100 | 100 | | |
| 45 | 120 | 22 | 10 | 10 | 10 | 10 | 100 | 100 | | |
| 46 | 100 | 8 | 8 | 8 | 8 | 8 | 100 | 100 | | |
| 47 | 140 | 20 | 10 | 10 | 10 | 10 | 100 | 100 | | |
| 48 | 150 | 13 | 10 | 10 | 10 | 10 | 100 | 100 | | |
| 49 | 100 | 15 | 10 | 10 | 10 | 10 | 100 | 100 | | |
| 50 | 100 | 19 | 10 | 10 | 10 | 10 | 100 | 100 | | |

**Appendix 3.2 Cont'd..: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media.**

| | mMLSB1 (ß-gur) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of ß-gur-positive/LPW(44°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | black | | | | | % | % | |
| 1 | 43 | 4 | 4 | 4 | 4 | 3 | 100 | 75 | *C.freundii* |
| 2 | 38 | 4 | 4 | 4 | 4 | 4 | 100 | 100 | |
| 3 | 41 | 2 | 2 | 2 | 2 | 2 | 100 | 100 | |
| 4 | 44 | 2 | 2 | 2 | 2 | 2 | 100 | 100 | |
| 5 | 30 | 2 | 2 | 2 | 2 | 1 | 100 | 50 | *E.coli* (ind -) |
| 6 | 29 | 4 | 4 | 4 | 4 | 4 | 100 | 100 | |
| 7 | 20 | 3 | 3 | 3 | 3 | 3 | 100 | 100 | |
| 8 | 25 | 3 | 3 | 3 | 2 | 2 | 100 | 67 | *Erwinia spp.* |
| 9 | 36 | 5 | 5 | 5 | 5 | 5 | 100 | 100 | |
| 10 | 27 | 4 | 4 | 4 | 4 | 4 | 100 | 100 | |

| | mMLSB1 (ß-gur) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of ß-gur-positive/LPW(44°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | black | | | | | % | % | |
| 11 | 40 | 2 | 2 | 2 | 2 | 2 | 100 | 100 | |
| 12 | 29 | 1 | 1 | 1 | 1 | 1 | 100 | 100 | |
| 13 | 30 | 2 | 2 | 2 | 2 | 2 | 100 | 100 | |
| 14 | 28 | 3 | 3 | 3 | 3 | 3 | 100 | 100 | |
| 15 | 31 | 3 | 3 | 3 | 3 | 2 | 100 | 67 | *E.coli* |
| 16 | 16 | 1 | 1 | 1 | 1 | 1 | 100 | 100 | |
| 17 | 25 | 3 | 3 | 3 | 3 | 3 | 100 | 100 | |
| 18 | 28 | 0 | | | | | | | |
| 19 | 11 | 2 | 2 | 2 | 2 | 2 | 100 | 100 | |
| 20 | 21 | 1 | 1 | 1 | 1 | 1 | 100 | 100 | |

**Appendix 3.2 Cont'd..: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media.**

| | mMLSB1 (ß-gur) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of ß-gur-positive/LPW(44°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | black | | | | | % | % | |
| 21 | 51 | 4 | 4 | 4 | 4 | 3 | 100 | 75 | *C.freundii* |
| 22 | 61 | 8 | 8 | 8 | 8 | 8 | 100 | 100 | |
| 23 | 17 | 5 | 5 | 5 | 5 | 2 | 100 | 40 | *C.freundii* (2)/*S.liquefaciens* |
| 24 | 8 | 2 | 2 | 2 | 2 | 2 | 100 | 100 | |
| 25 | 18 | 5 | 5 | 5 | 5 | 2 | 100 | 40 | *C.freundii* (2)/*E.cloacae* |
| 26 | 8 | 2 | 2 | 2 | 2 | 0 | 100 | 0 | *E.cloacae*/*C.freundii* |
| 27 | 48 | 4 | 4 | 4 | 4 | 4 | 100 | 100 | |
| 28 | 40 | 4 | 4 | 4 | 3 | 2 | 100 | 50 | *C.freundii* (2) |
| 29 | 42 | 3 | 3 | 3 | 3 | 2 | 100 | 67 | *C.freundii* |
| 30 | 47 | 5 | 5 | 5 | 5 | 3 | 100 | 60 | *C.freundii* /*E.cloacae* |

| | mMLSB1 (ß-gur) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of ß-gur-positive/LPW(44°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | black | | | | | % | % | |
| 31 | 40 | 4 | 4 | 4 | 4 | 4 | 100 | 100 | |
| 32 | 23 | 1 | 1 | 1 | 1 | 1 | 100 | 100 | |
| 33 | 13 | 2 | 2 | 2 | 2 | 2 | 100 | 100 | |
| 34 | 29 | 3 | 3 | 3 | 3 | 3 | 100 | 100 | |
| 35 | 14 | 1 | 1 | 1 | 1 | 1 | 100 | 100 | |
| 36 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 37 | 33 | 2 | 2 | 2 | 2 | 2 | 100 | 100 | |
| 38 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 39 | 25 | 1 | 1 | 1 | 1 | 1 | 100 | 100 | |
| 40 | 31 | 1 | 1 | 1 | 1 | 1 | 100 | 100 | |

| Appendix 3.2 Cont'd..: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mMLSB1 (ß-gur) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of ß-gur-positive/LPW(44°C)-negative organisms | |
| | total | black | | | | | % | % | | |
| 41 | 25 | 5 | 5 | 5 | 5 | 5 | 100 | 100 | | |
| 42 | 17 | 4 | 4 | 4 | 4 | 4 | 100 | 100 | | |
| 43 | 17 | 2 | 2 | 2 | 2 | 2 | 100 | 100 | | |
| 44 | 21 | 4 | 4 | 4 | 4 | 4 | 100 | 100 | | |
| 45 | 120 | 14 | 10 | 9 | 9 | 10 | 90 | 100 | *E.coli* | |
| 46 | 100 | 3 | 3 | 3 | 3 | 3 | 100 | 100 | | |
| 47 | 140 | 11 | 10 | 10 | 10 | 10 | 100 | 100 | | |
| 48 | 150 | 8 | 8 | 8 | 8 | 8 | 100 | 100 | | |
| 49 | 100 | 10 | 10 | 10 | 10 | 9 | 100 | 90 | *E.cloacae* | |
| 50 | 100 | 17 | 10 | 10 | 10 | 9 | 100 | 90 | *Salmonella arizonae* | |

**Appendix 3.2 Cont'd..: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media.**

| | mMLSB2 (alpha-gal) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of alpha-gal-positive/LPW(37°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | blue | | | | | % | % | |
| 1 | 53 | 46 | 10 | 10 | 10 | 1 | 100 | 10 | |
| 2 | 37 | 20 | 10 | 9 | 9 | 3 | 90 | 30 | *E.cloacae* |
| 3 | 46 | 21 | 10 | 10 | 10 | 3 | 100 | 30 | |
| 4 | 50 | 26 | 10 | 10 | 10 | 4 | 100 | 40 | |
| 5 | 40 | 17 | 10 | 8 | 7 | 3 | 80 | 30 | *E.cloacae* (2) |
| 6 | 15 | 7 | 7 | 7 | 6 | 1 | 100 | 14 | |
| 7 | 20 | 11 | 10 | 9 | 9 | 1 | 90 | 10 | *E. sakazakii* |
| 8 | 6 | 4 | 4 | 3 | 3 | 2 | 75 | 50 | *E.cloacae* |
| 9 | 5 | 5 | 5 | 5 | 4 | 2 | 100 | 40 | |
| 10 | 1 | 1 | 1 | 1 | 1 | 0 | 100 | 0 | |

| | mMLSB2 (alpha-gal) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of alpha-gal-positive/LPW(37°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | blue | | | | | % | % | |
| 11 | 29 | 11 | 10 | 10 | 10 | 5 | 100 | 50 | |
| 12 | 25 | 13 | 10 | 10 | 9 | 4 | 100 | 40 | |
| 13 | 30 | 19 | 10 | 10 | 10 | 4 | 100 | 40 | |
| 14 | 20 | 15 | 10 | 10 | 10 | 4 | 100 | 40 | |
| 15 | 25 | 15 | 10 | 10 | 10 | 6 | 100 | 60 | |
| 16 | 26 | 10 | 10 | 10 | 10 | 0 | 100 | 0 | |
| 17 | 30 | 16 | 10 | 10 | 9 | 5 | 100 | 50 | |
| 18 | 24 | 18 | 10 | 10 | 10 | 2 | 100 | 20 | |
| 19 | 20 | 12 | 10 | 10 | 10 | 4 | 100 | 40 | |
| 20 | 19 | 12 | 10 | 10 | 10 | 3 | 100 | 30 | |

**Appendix 3.2 Cont'd..: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media.**

| | mMLSB2 (alpha-gal) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of alpha-gal-positive/LPW(37°C)-negative organisms |
|---|---|---|---|---|---|---|---|---|---|
| | total | blue | | | | | % | % | |
| 21 | 8 | 3 | 3 | 3 | 3 | 1 | 100 | 33 | |
| 22 | 7 | 6 | 6 | 6 | 6 | 0 | 100 | 0 | |
| 23 | 10 | 8 | 8 | 7 | 7 | 3 | 88 | 38 | *C.freundii* |
| 24 | 13 | 10 | 10 | 10 | 10 | 1 | 100 | 10 | |
| 25 | 19 | 14 | 10 | 10 | 10 | 6 | 100 | 60 | |
| 26 | 7 | 6 | 6 | 6 | 6 | 1 | 100 | 17 | |
| 27 | 63 | 45 | 10 | 10 | 10 | 2 | 100 | 20 | |
| 28 | 53 | 40 | 10 | 9 | 9 | 2 | 90 | 20 | *P.mirabilis* |
| 29 | 54 | 43 | 10 | 10 | 10 | 1 | 100 | 10 | |
| 30 | 36 | 18 | 10 | 10 | 10 | 2 | 100 | 20 | |
| | | | | | | | | | |
| | mMLSB2 (alpha-gal) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of alpha-gal-positive/LPW(37°C)-negative organisms |
| | total | blue | | | | | % | % | |
| 31 | 22 | 22 | 10 | 10 | 10 | 3 | 100 | 30 | |
| 32 | 20 | 20 | 10 | 10 | 10 | 4 | 100 | 40 | |
| 33 | 31 | 28 | 10 | 10 | 10 | 2 | 100 | 20 | |
| 34 | 28 | 27 | 10 | 10 | 9 | 3 | 100 | 30 | |
| 35 | 17 | 15 | 10 | 10 | 8 | 2 | 100 | 20 | |
| 36 | 19 | 17 | 10 | 10 | 8 | 5 | 100 | 50 | |
| 37 | 28 | 27 | 10 | 9 | 9 | 1 | 90 | 10 | *K.pneumoniae* |
| 38 | 29 | 28 | 10 | 10 | 9 | 2 | 100 | 20 | |
| 39 | 24 | 23 | 10 | 10 | 9 | 3 | 100 | 30 | |
| 40 | 30 | 28 | 10 | 10 | 10 | 3 | 100 | 30 | |

| Appendix 3.2 Cont'd..: Counts of membraned filtered water samples 1 -20 (simulated chlorine-damaged river water) on 4 test media. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mMLSB2 (alpha-gal) | | OX - | LPW (37°C) | LPW (44°C) | TW (44°C) | confirmed coliform | confirmed *E.coli* | Identification of alpha-gal-positive/LPW(37°C)-negative organisms | |
| | total | blue | | | | | % | % | | |
| 41 | 20 | 2 | 2 | 2 | 2 | 2 | 100 | 100 | | |
| 42 | 162 | 8 | 8 | 8 | 8 | 8 | 100 | 100 | | |
| 43 | 44 | 7 | 7 | 7 | 7 | 7 | 100 | 100 | | |
| 44 | 55 | 5 | 5 | 5 | 5 | 5 | 100 | 100 | | |
| 45 | 123 | 17 | 10 | 10 | 10 | 10 | 100 | 100 | | |
| 46 | 135 | 18 | 10 | 10 | 10 | 10 | 100 | 100 | | |
| 47 | 142 | 15 | 10 | 10 | 10 | 10 | 100 | 100 | | |
| 48 | 159 | 13 | 10 | 9 | 9 | 10 | 90 | 90 | *E.coli* | |
| 49 | 160 | 14 | 10 | 10 | 10 | 10 | 100 | 100 | | |
| 50 | 200 | 17 | 10 | 10 | 10 | 10 | 100 | 100 | | |

| Appendix 4.1: Fluorescence values of coumarin core molecules (0.33 mmol $l^{-1}$) over the pH range 4.0 - 8.0. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **4** | **4.5** | **5** | **5.5** | **6** | **6.5** | **7** | **7.5** | **8** | |
| 7-hydroxy-4-methylcoumarin (4-MU) | 6893 | 5781 | 7308 | 9065 | 8419 | 16379 | 27943 | 49713 | 53879 | |
| methyl 7-hydroxycoumarin-4-acetate | 12532 | 14561 | 19008 | 46812 | 55577 | 67837 | 71328 | 71936 | 72256 | |
| 3-butyl-7-hydroxy-4-methylcoumarin | 6205 | 6159 | 6257 | 6348 | 7581 | 9824 | 15052 | 27965 | 46570 | |
| 7-hydroxycoumarin-4-acetic acid | 13841 | 13462 | 13658 | 14302 | 16829 | 22396 | 31823 | 49668 | 62804 | |
| 6-chloro-7-hydroxy-4-methyl-3-octylcoumarin | 256 | 266 | 391 | 678 | 1065 | 1914 | 2591 | 3473 | 3882 | |
| 4-benzyl-7-hydroxycoumarin | 125 | 162 | 122 | 168 | 168 | 174 | 195 | 424 | 513 | |
| 3-acetyl-6-hexyl-7-hydroxy-4-methylcoumarin | 4572 | 3354 | 4850 | 4651 | 4432 | 4828 | 5146 | 5707 | 4673 | |
| ethyl 7-hydroxycoumarin-3-carboxylate | 58241 | 47279 | 53642 | 61074 | 63833 | 67486 | 70043 | 68078 | 64956 | |
| 3-chloro-7-hydroxy-4-methylcoumarin | 10523 | 10065 | 9418 | 11018 | 8411 | 13331 | 20021 | 33657 | 44858 | |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 137 | 143 | 137 | 146 | 229 | 232 | 189 | 156 | 180 | |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 308 | 327 | 409 | 601 | 1157 | 2625 | 7056 | 19344 | 38025 | |
| 6-chloro-7-hydroxy-4-methylcoumarin | 1001 | 1138 | 2130 | 4981 | 9919 | 23497 | 43287 | 65322 | 65224 | |
| methyl 7-hydroxycoumarin-3-carboxylate | 49741 | 51036 | 50425 | 53321 | 57484 | 70901 | 76883 | 78375 | 74795 | |
| 6-hexyl-7-hydroxy-4-methylcoumarin | 4242 | 4139 | 4547 | 4325 | 4248 | 4465 | 4743 | 4761 | 3839 | |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 1221 | 1361 | 2396 | 5405 | 10310 | 23128 | 47880 | 64248 | 65145 | |
| ethyl 4,8-dimethyl-6-ethyl-7-hydroxycoumarin-3-propanoate | 562 | 641 | 769 | 751 | 1135 | 1050 | 1367 | 2161 | 3437 | |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 6385 | 6537 | 6623 | 6366 | 7529 | 8344 | 11576 | 21532 | 38803 | |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 763 | 818 | 1132 | 1978 | 3604 | 7111 | 11356 | 21892 | 36343 | |
| 7-hydroxycoumarin-3-carboxylic acid | 56041 | 44828 | 37814 | 34756 | 43979 | 48902 | 56175 | 65600 | 67104 | |
| 7-hydroxy-3-methoxyacetylcoumarin | 15996 | 16365 | 17149 | 18117 | 19826 | 21749 | 23424 | 22447 | 22188 | |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 104 | 229 | 98 | 137 | 146 | 168 | 186 | 211 | 238 | |
| 7-hydroxy-4-methyl-3-propionic acid | 8466 | 10114 | 7880 | 11363 | 10749 | 15846 | 20564 | 28228 | 59068 | |

| **Appendix 4.2: The effect of each coumarin core molecule (0.5 mmol $l^{-1}$) on growth - increase in absorbance (690 nm) over 300m whilst incubating at 37°C.** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* NCTC 10418 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | 0 | -0.002 | -0.001 | -0.001 | 0.003 | 0.007 | 0.008 | 0.017 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | -0.001 | 0 | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.004 | 0.009 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.004 | -0.005 | -0.004 | -0.003 | -0.003 | -0.002 | -0.002 | -0.002 | -0.002 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | 0.001 | 0.005 | 0.011 | 0.028 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | 0.002 | 0.005 | 0.005 | 0.005 | 0.006 | 0.008 | 0.009 | 0.014 | 0.028 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | 0.001 | 0.004 | 0.007 | 0.018 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.001 | 0 | 0 | 0 | 0 | 0 | 0 | 1E-03 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | -0.003 | 0.001 | 0 | -0.001 | 0 | 0 | 0.001 | 0.001 | 0.002 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | 0.001 | 0.001 | 0.003 | 0.004 | 0.008 | 0.009 | 0.009 | 0.007 | 0.017 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -1E-03 | -1E-03 | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.002 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0 | -0.002 | -0.001 | -0.002 | -0.001 | -0.001 | -0.001 | -0.001 | -0.001 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.017 | -0.018 | -0.018 | -0.019 | -0.018 | -0.018 | -0.018 | -0.017 | -0.018 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.061 | -0.197 | -0.18 | -0.195 | -0.175 | -0.177 | -0.178 | -0.186 | -0.196 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.011 | 0.01 | 0.01 | 0.011 | 0.014 | 0.015 | 0.017 | 0.02 | 0.026 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | 0.001 | 0.005 | 0.009 | 0.024 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | -0.001 | 0.001 | 0.005 | 0.008 | 0.02 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | -0.003 | -0.003 | -0.003 | -0.003 | -0.003 | -0.002 | 0.007 | -0.001 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | 0 | -0.001 | -0.001 | -0.001 | 0 | 0.002 | 0.006 | 0.011 | 0.028 |
| Control (growth medium and solvent) | 0 | 0 | -0.001 | 0 | -0.001 | -0.001 | 0.001 | 0.003 | 0.008 | 0.012 | 0.029 |
| | | | | | | | | | | | |
| *E. coli* FRHECO1 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | 0.001 | 0.005 | 0.009 | 0.024 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0.001 | 0.002 | 0.007 | 0.014 | 0.031 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.007 | -0.008 | -0.008 | -0.007 | -0.002 | -0.006 | -0.003 | 0 | 0.016 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | -0.001 | 0 | -0.001 | 0 | 0.001 | 0.003 | 0.01 | 0.017 | 0.037 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | -0.004 | -0.005 | -0.007 | -0.01 | -0.008 | -0.006 | -0.004 | 1E-03 | 0.018 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | -0.001 | -0.001 | -0.002 | -0.001 | -0.001 | 0.001 | 0.007 | 0.016 | 0.034 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | -0.002 | -0.002 | -0.001 | 0 | 0.001 | 0.004 | 0.009 | 0.021 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | -0.001 | 0 | -0.001 | 0 | 0 | 0.001 | 0.004 | 0.007 | 0.016 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | 0 | 0.004 | 0.008 | 0.009 | 0.01 | 0.02 | 0.022 | 0.028 | 0.036 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | -0.002 | -0.002 | -0.002 | -0.001 | 0.001 | 0.005 | 0.011 | 0.026 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0.004 | 0.002 | 0.003 | 0.001 | 0.001 | 0 | 0.002 | 0.002 | 0.003 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.009 | -0.008 | -0.008 | -0.008 | -0.008 | -0.007 | -0.005 | -0.003 | 0.002 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.037 | -0.072 | -0.064 | -0.068 | -0.194 | -0.187 | -0.186 | -0.197 | -0.191 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.003 | 0.006 | 0.009 | 0.009 | 0.009 | 0.012 | 0.016 | 0.021 | 0.043 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | -0.001 | 0 | 0 | 0 | 0.001 | 0.004 | 0.011 | 0.019 | 0.048 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | 0.004 | 0.004 | 0.004 | 0.004 | 0.005 | 0.008 | 0.015 | 0.023 | 0.054 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.004 | 0.011 | 0.019 | 0.049 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | 1E-03 | 1E-03 | 1E-03 | 1E-03 | 0.002 | 0.004 | 0.012 | 0.021 | 0.053 |
| Control (growth medium and solvent) | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.006 | 0.013 | 0.022 | 0.053 |

| Appendix 4.2 (Cont'd..): The effect of each coumarin core molecule (0.5 mmol $l^{-1}$) on growth - increase in absorbance (690 nm) over 300m whilst incubating at 37°C. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* FRHECO2 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | -0.001 | 0.001 | 0.006 | 0.012 | 0.032 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | -0.005 | -0.005 | -0.005 | -0.004 | -0.003 | 0 | 0.009 | 0.022 | 0.063 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.006 | -0.007 | -0.006 | -0.006 | -0.005 | -0.002 | 0.006 | 0.015 | 0.05 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | -0.004 | -0.003 | -0.004 | -0.003 | -0.001 | 0.002 | 0.011 | 0.024 | 0.066 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | 0 | -0.004 | -0.003 | -1E-03 | 0 | 0.006 | 0.007 | 0.024 | 0.061 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | -0.001 | -0.001 | -0.002 | -0.001 | 0 | 0.004 | 0.015 | 0.029 | 0.077 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.002 | 0.002 | 0.001 | 0 | 0.002 | 0.004 | 0.01 | 0.017 | 0.048 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | 0.003 | 0.009 | 0.016 | 0.047 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | 0.004 | 0.006 | 0.009 | 0.012 | 0.017 | 0.021 | 0.027 | 0.038 | 0.16 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.003 | 0.005 | 0.006 | 0.006 | 0.007 | 0.01 | 0.019 | 0.028 | 0.066 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0.002 | 1E-03 | 1E-03 | 1E-03 | 0.002 | 0.004 | 0.011 | 0.02 | 0.056 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.004 | -0.005 | -0.005 | -0.005 | -0.004 | -0.001 | 0.007 | 0.019 | 0.06 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.052 | -0.055 | -0.044 | -0.048 | -0.043 | -0.042 | -0.028 | -0.025 | 0.007 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.006 | 0.008 | 0.01 | 0.011 | 0.013 | 0.018 | 0.028 | 0.042 | 0.09 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.002 | 0.004 | 0.008 | 0.02 | 0.036 | 0.087 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | 0.003 | 0.002 | 0.002 | 0.002 | 0.004 | 0.007 | 0.019 | 0.033 | 0.079 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0 | 0.003 | 0.006 | 0.018 | 0.034 | 0.081 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | 1E-03 | 1E-03 | 0 | 1E-03 | 0.002 | 0.006 | 0.017 | 0.032 | 0.078 |
| Control (growth medium and solvent) | 0 | 0 | 0.002 | 0.002 | 0.002 | 0.002 | 0.004 | 0.008 | 0.021 | 0.036 | 0.085 |
| | | | | | | | | | | | |
| *E. coli* FRHECO3 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0.001 | 0.001 | 0.004 | 0.005 | 0.002 | 0.006 | 0.009 | 0.018 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.003 | 0.005 | 0.01 | 0.016 | 0.037 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.004 | -0.005 | -0.005 | -0.005 | -0.003 | -0.002 | 0.003 | 0.008 | 0.025 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.002 | 0.003 | 0.004 | 0.01 | 0.017 | 0.038 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | -0.001 | -0.003 | -0.003 | -0.005 | -0.004 | -0.004 | 0.003 | 0.005 | 0.026 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.004 | 0.008 | 0.013 | 0.027 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.001 | 0.003 | 0.007 | 0.011 | 0.024 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.005 | 0.007 | 0.014 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | -0.001 | 1E-03 | 0.005 | 0.002 | 0.004 | 0.005 | 0.012 | 0.014 | 0.025 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.003 | 0.008 | 0.014 | 0.031 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | -0.002 | 0 | 0.001 | 0 | -0.001 | 0.003 | 0.008 | 0.01 | 0.028 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.013 | -0.013 | -0.013 | -0.014 | -0.013 | -0.011 | -0.003 | 0.004 | 0.03 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.008 | 0.001 | 0.009 | -0.009 | -1E-03 | 0.003 | 0.03 | 0.015 | 0.042 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.004 | 0.007 | 0.012 | 0.01 | 0.013 | 0.016 | 0.028 | 0.036 | 0.068 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0.001 | 0.004 | 0.013 | 0.023 | 0.058 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | 0.001 | 0 | 0 | 0 | 0.002 | 0.004 | 0.014 | 0.023 | 0.056 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0 | 1E-03 | 0.004 | 0.014 | 0.023 | 0.053 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | 0.003 | 0.01 | 0.019 | 0.047 |
| Control (growth medium and solvent) | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.002 | 0 | 0.003 | 0.012 | 0.021 | 0.053 |

| Appendix 4.2 (Cont'd..): The effect of each coumarin core molecule (0.5 mmol $l^{-1}$) on growth - increase in absorbance (690 nm) over 300m whilst incubating at 37°C. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *K. pneumoniae* NCTC 10896 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | -0.001 | -0.002 | -0.001 | 0 | 0.003 | 0.002 | 1E-03 | 0.004 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | -0.002 | -0.002 | -0.003 | -0.003 | -0.003 | -0.002 | -1E-03 | 0.001 | 0.006 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | -0.002 | -0.003 | -0.003 | -0.003 | -0.003 | -0.003 | -0.003 | -0.003 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | 0 | 0.001 | 0 | 0.001 | 0.001 | 0.004 | 0.004 | 0.005 | 0.01 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | 0.016 | 0.008 | 0.002 | 0.007 | 0.004 | -0.001 | -0.002 | 1E-03 | 0.003 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | -0.001 | 0.001 | 0 | 0 | 0 | 0.001 | 0.002 | 0.003 | 0.006 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | 0.001 | -0.003 | -0.003 | -0.003 | -0.002 | -0.003 | -0.002 | -0.003 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | 0 | 0 | -0.002 | -0.002 | -0.002 | -0.002 | -0.002 | -0.002 | -0.001 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | 0.004 | 0.005 | 0.004 | 0.007 | 0.008 | 0.008 | 0.011 | 0.01 | 0.012 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | 0.002 | 0.007 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0.002 | 0.001 | 0.001 | 0.002 | 0.001 | 0.002 | 0.002 | 0.002 | 0.005 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.012 | -0.013 | -0.012 | -0.012 | -0.012 | -0.012 | -0.011 | -0.011 | -0.007 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.02 | -0.026 | -0.04 | -0.032 | -0.027 | -0.03 | -0.035 | -0.033 | -0.042 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.005 | 0.007 | 0.007 | 0.01 | 0.011 | 0.012 | 0.012 | 0.015 | 0.019 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | -0.001 | -0.001 | 0 | 0 | 0.001 | 0.001 | 0.004 | 0.007 | 0.015 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | -0.001 | -0.001 | 0 | 0 | 0 | 0.001 | 0.003 | 0.003 | 0.007 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.001 | -0.001 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | -0.001 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.002 | 0.003 | 0.004 | 0.006 | 0.009 | 0.015 |
| Control (growth medium and solvent) | 0 | 0 | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.003 | 0.005 | 0.008 | 0.016 |
| | | | | | | | | | | | |
| *K. pneumoniae* FRHKPN1 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | 0.003 | 0.012 | 0.024 | 0.058 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | -0.002 | -0.002 | -0.003 | -0.002 | 0 | 0.004 | 0.017 | 0.034 | 0.085 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.007 | -0.007 | -0.008 | -0.007 | -0.005 | -1E-03 | 0.009 | 0.025 | 0.074 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | 0.001 | -0.001 | 0.003 | 0.004 | 0.005 | 0.011 | 0.025 | 0.045 | 0.099 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | -1E-03 | -0.002 | -0.007 | -0.006 | -0.005 | 0 | 0.015 | 0.032 | 0.087 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0 | -0.001 | -0.001 | -0.001 | 0.001 | 0.005 | 0.017 | 0.04 | 0.087 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.001 | -0.001 | 0 | 0.001 | 0.004 | 0.014 | 0.026 | 0.066 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | -0.001 | 0 | -0.002 | -0.001 | 0 | 0.002 | 0.01 | 0.02 | 0.053 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | 0.004 | 0.009 | 0.007 | 0.011 | 0.01 | 0.014 | 0.025 | 0.04 | 0.089 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.001 | 0.001 | 0 | 0.001 | 0.003 | 0.007 | 0.019 | 0.037 | 0.087 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0 | 0.003 | 0.001 | 0.001 | 0.005 | 0.006 | 0.018 | 0.037 | 0.084 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.012 | -0.013 | -0.011 | -0.011 | -0.009 | -0.004 | 0.01 | 0.029 | 0.088 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.104 | -0.089 | -0.109 | -0.098 | -0.102 | -0.115 | -0.11 | -0.087 | -0.031 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.002 | 0.008 | 0.008 | 0.011 | 0.014 | 0.018 | 0.032 | 0.051 | 0.112 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.004 | 0.009 | 0.024 | 0.047 | 0.109 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | 0 | 0 | 0.002 | 0.006 | 0.005 | 0.009 | 0.024 | 0.045 | 0.104 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | -0.002 | -0.002 | 0 | 0.002 | 0.005 | 0.02 | 0.041 | 0.099 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | 0.001 | 0.001 | 0.003 | 0.004 | 0.005 | 0.01 | 0.025 | 0.045 | 0.102 |
| Control (growth medium and solvent) | 0 | 0 | 0 | 0.001 | 0.002 | 0.002 | 0.004 | 0.009 | 0.025 | 0.046 | 0.106 |

| Appendix 4.2 (Cont'd..): The effect of each coumarin core molecule (0.5 mmol $l^{-1}$) on growth - increase in absorbance (690 nm) over 300m whilst incubating at 37°C. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *K. pneumoniae* FRHKPN2 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.003 | 0.007 | 0.017 | 0.048 | 0.107 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | -0.001 | 0 | 0.001 | 0.002 | 0.008 | 0.01 | 0.025 | 0.073 | 0.145 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | 0.001 | 0.001 | 0.003 | 0.007 | 0.01 | 0.022 | 0.06 | 0.133 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.008 | 0.011 | 0.026 | 0.076 | 0.149 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | -0.002 | 0 | 0 | 0.002 | 0.004 | 0.009 | 0.023 | 0.068 | 0.14 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.003 | 0.007 | 0.019 | 0.054 | 0.116 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | 0.001 | 0.001 | 0.001 | 0.003 | 0.007 | 0.017 | 0.046 | 0.106 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | 0 | 0.001 | 0.001 | 0.003 | 0.006 | 0.009 | 0.019 | 0.049 | 0.105 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | 0.003 | 0.002 | 0.003 | 0.007 | 0.01 | 0.014 | 0.028 | 0.064 | 0.132 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | 0 | 0 | 0.002 | 0.008 | 0.01 | 0.023 | 0.066 | 0.134 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0.002 | -0.001 | -0.001 | 0 | 0 | 0.004 | 0.012 | 0.031 | 0.074 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 1E-03 | -0.002 | -1E-03 | -1E-03 | 0 | 0.007 | 0.018 | 0.056 | 0.12 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 1E-03 | -0.002 | 0 | -0.001 | 0 | 0.003 | 0.018 | 0.055 | 0.12 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.002 | 0.001 | 0.003 | 0.005 | 0.006 | 0.012 | 0.026 | 0.068 | 0.146 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | 0.001 | 0 | 0 | 0.001 | 0.009 | 0.01 | 0.026 | 0.074 | 0.15 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | 0.002 | 0 | 0 | 1E-03 | 0.006 | 0.009 | 0.024 | 0.068 | 0.141 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | -0.001 | 0 | 1E-03 | 0.003 | 0.009 | 0.022 | 0.06 | 0.128 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | 0.002 | 0 | 0.001 | 0.002 | 0.004 | 0.01 | 0.023 | 0.065 | 0.146 |
| Control (growth medium and solvent) | 0 | 0 | 0.001 | -0.001 | 0 | 0.001 | 0.005 | 0.009 | 0.023 | 0.067 | 0.144 |
| | | | | | | | | | | | |
| *K. pneumoniae* FRHKPN3 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.004 | 0.01 | 0.028 | 0.061 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | 0 | 0 | 0.002 | 0 | 0.002 | 0.007 | 0.017 | 0.049 | 0.11 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.005 | 0.009 | 0.02 | 0.052 | 0.117 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.006 | 0.009 | 0.024 | 0.066 | 0.139 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | -0.001 | -0.002 | 0 | -0.001 | 0.002 | 0.007 | 0.022 | 0.064 | 0.144 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0 | 0 | 0.001 | 0.001 | 0.004 | 0.009 | 0.022 | 0.064 | 0.137 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.003 | 0.006 | 0.012 | 0.035 | 0.087 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | -0.001 | -0.003 | -0.003 | -0.002 | 0 | 0.004 | 0.013 | 0.04 | 0.09 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | 0.002 | 0.003 | 0.004 | 0.007 | 0.008 | 0.011 | 0.019 | 0.051 | 0.233 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.002 | 0.003 | 0.008 | 0.02 | 0.053 | 0.112 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | -0.001 | 0 | -0.005 | -0.002 | 0 | -0 | -0 | 0.003 | 0.011 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | -0.001 | -0.001 | 0 | 0.003 | 0.008 | 0.023 | 0.058 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.009 | -0.007 | -0.006 | 0 | 0.002 | 0.005 | 0.009 | 0.043 | 0.112 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0.002 | 0.003 | 0.005 | 0.007 | 0.011 | 0.025 | 0.067 | 0.151 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.008 | 0.011 | 0.026 | 0.075 | 0.161 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | -0.001 | -0.001 | -0.001 | 0 | 0 | 0.009 | 0.025 | 0.075 | 0.156 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.009 | 0.022 | 0.063 | 0.138 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | -0.001 | -0.001 | -0.001 | 0 | 0.003 | 0.009 | 0.025 | 0.073 | 0.157 |
| Control (growth medium and solvent) | 0 | 0 | 0 | 0 | 0 | 0.002 | 0.009 | 0.01 | 0.026 | 0.075 | 0.172 |

| Appendix 4.2 (Cont'd..): The effect of each coumarin core molecule (0.5 mmol $l^{-1}$) on growth - increase in absorbance (690 nm) over 300m whilst incubating at 37°C. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. freundii* NCTC 9750 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.003 | 0.005 | 0.011 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | 0 | 0 | -0.001 | 0 | 0 | 0.001 | 0.003 | 0.007 | 0.017 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | -1E-03 | 0.001 | 0.001 | 0.001 | 0.004 | 0.008 | 0.019 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | -0.002 | -0.001 | 1E-03 | 0.002 | 0.001 | 1E-03 | 1E-03 | 0.005 | 0.02 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | -1E-03 | -0.002 | -0.002 | -0.002 | 0 | -1E-03 | 0 | 0.005 | 0.012 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.005 | 0.014 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1E-03 | 1E-03 | 0 | 0.003 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | 0.001 | 0.001 | 0 | 0.002 | 0 | 0.001 | 0.005 | 0.013 | 0.032 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | 0 | 0.002 | 0.003 | 0.005 | 0.004 | 0.004 | 0.013 | 0.014 | 0.023 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | -0.001 | 0 | 0 | 0 | 0.003 | 0.006 | 0.015 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0 | -0.001 | 0 | -0.001 | 0 | 1E-03 | 0 | 0.003 | 0.011 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 1E-03 | -0.001 | 0.001 | 0.002 | 1E-03 | 0.004 | 0.013 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | -0.004 | -1E-03 | -0.002 | 0 | -0.005 | -0.003 | 0 | 0.008 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.002 | 0.003 | 0.003 | 0.004 | 0.004 | 0.005 | 0.007 | 0.012 | 0.024 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | 0 | 0 | 0.001 | -0.001 | 0 | 0.002 | 0.002 | 0.007 | 0.017 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | -0.001 | -0.001 | 0.001 | -0.001 | 0 | 0.002 | 0.001 | 0.004 | 0.013 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.002 | 0.002 | 0.002 | -0.001 | 0 | 0.002 | 0.001 | 0.005 | 0.014 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | 0 | -0.001 | 0 | -0.001 | 0 | 0.001 | 0.001 | 0.005 | 0.016 |
| Control (growth medium and solvent) | 0 | 0 | 0 | -0.001 | 0.001 | 0 | 0 | 0.002 | 0.002 | 0.006 | 0.026 |
| | | | | | | | | | | | |
| *C. freundii* FRHCFR1 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.003 | 0.007 | 0.02 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.005 | 0.012 | 0.027 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.004 | 0.01 | 0.025 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | 1E-03 | 1E-03 | 1E-03 | 1E-03 | 0.001 | 0.002 | 0.005 | 0.014 | 0.03 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | 0 | -1E-03 | -1E-03 | 0 | 0 | 0.001 | 0.002 | 0.009 | 0.024 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.002 | 0.004 | 0.01 | 0.029 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | 0.002 | 0.002 | 0.006 | 0.013 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | 0.001 | 0.002 | 0.005 | 0.005 | 0.006 | 0.007 | 0.008 | 0.011 | 0.022 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | 0 | -0.003 | 1E-03 | 0.003 | 0.003 | 0.003 | 0.006 | 0.012 | 0.036 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.001 | 0.002 | 0.001 | 0.001 | 0.002 | 0.003 | 0.005 | 0.012 | 0.024 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | 0.001 | 0.003 | 0.009 | 0.026 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.003 | 0.009 | 0.026 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.002 | 0.001 | 0.002 | 0 | -0.001 | 0 | 0.002 | 0.019 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.003 | 0.003 | 0.005 | 0.007 | 0.006 | 0.006 | 0.008 | 0.014 | 0.03 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | 0 | -0.001 | 0 | 0 | 0 | 0.001 | 0.004 | 0.011 | 0.028 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | 0 | -0.001 | 0.001 | 0.002 | 0.002 | 0.003 | 0.005 | 0.011 | 0.024 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 1E-03 | 0.001 | 0.002 | 0.004 | 0.01 | 0.022 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.008 | 0.021 |
| Control (growth medium and solvent) | 0 | 0 | 0 | -0.001 | -0.001 | -0.001 | 0 | 0 | 0.002 | 0.007 | 0.019 |

| Appendix 4.2 (Cont'd..): The effect of each coumarin core molecule (0.5 mmol $l^{-1}$) on growth - increase in absorbance (690 nm) over 300m whilst incubating at 37°C. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. freundii* FRHCFR2 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.001 | 0 | 0 | 0 | 0.003 | 0.007 | 0.017 | 0.042 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | -0.001 | 0 | 0.002 | 0.002 | 0.003 | 0.006 | 0.012 | 0.028 | 0.071 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.002 | 0.003 | 0.005 | 0.009 | 0.02 | 0.048 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.004 | 0.005 | 0.012 | 0.032 | 0.075 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | -0.002 | -0.003 | -0.003 | -0.003 | 0 | 0.001 | 0.009 | 0.029 | 0.068 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.005 | 0.012 | 0.03 | 0.063 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | -1E-03 | 0 | -0.004 | -0.002 | -0.002 | 0.004 | 0.01 | 0.019 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.004 | 0.008 | 0.02 | 0.043 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | 0 | 0 | 0.002 | 0.006 | 0.006 | 0.008 | 0.012 | 0.025 | 0.055 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0.001 | 0 | 0 | 0.003 | 0.007 | 0.019 | 0.045 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 0.001 | 0 | -0.001 | 0.001 | 0.001 | 0.001 | 0.006 | 0.014 | 0.034 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | -0.003 | -0.002 | 0.001 | 0.001 | 0.006 | 0.023 | 0.056 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.005 | 0.002 | 1E-03 | 0.004 | 0.004 | 0.005 | 0.01 | 0.031 | 0.067 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.004 | 0.005 | 0.005 | 0.008 | 0.005 | 0.011 | 0.017 | 0.037 | 0.073 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | -0.001 | -0.001 | -0.001 | 0 | 0.002 | 0.005 | 0.011 | 0.03 | 0.071 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | 0 | 0 | 0 | 0.003 | 0.002 | 0.003 | 0.009 | 0.028 | 0.065 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -1E-03 | -1E-03 | -1E-03 | -1E-03 | 0.002 | 0.003 | 0.007 | 0.022 | 0.059 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | 0 | 0 | 0 | 0 | 0.002 | 0.003 | 0.009 | 0.028 | 0.066 |
| Control (growth medium and solvent) | 0 | 0 | 0 | -0.001 | 0 | 0 | 0.001 | 0.003 | 0.009 | 0.027 | 0.068 |
| | | | | | | | | | | | |
| *C. freundii* FRHCFR3 | | | | | | | | | | | |
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
| 7-hydroxy-4-methylcoumarin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.002 | 0.006 | 0.016 | 0.149 |
| methyl 7-hydroxycoumarin-4-acetate | 0 | 0 | 0 | 0 | 0 | 0 | 0.004 | 0.005 | 0.011 | 0.042 | 0.16 |
| 3-butyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.002 | -0.001 | -0.001 | -0.001 | 0 | 0.002 | 0.003 | 0.013 | 0.07 |
| 7-hydroxycoumarin-4-acetic acid | 0 | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.003 | 0.007 | 0.015 | 0.049 | 0.168 |
| 4-benzyl-7-hydroxycoumarin | 0 | 0 | 0.004 | 0.005 | 0.005 | 0.005 | 0.005 | 0.006 | 0.015 | 0.029 | 0.105 |
| ethyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | -0.001 | -0.001 | -0.001 | 0 | 0 | 0.003 | 0.018 | 0.041 | 0.098 |
| 3-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.003 | -0.002 | -0.002 | -0.002 | 0 | -1E-03 | 0.002 | 0.01 | 0.041 |
| ethyl 6-chloro-7-hydroxy-8-methylcoumarin-3-carboxylate | 0 | 0 | -0.002 | -0.002 | -0.002 | -0.002 | 0.001 | 0.002 | 0.012 | 0.036 | 0.075 |
| 6-chloro-3,4-cyclohexeno-7-hydroxycoumarin | 0 | 0 | -0.033 | -0.033 | -0.03 | -0.026 | -0.027 | -0.028 | -0.02 | 0.031 | 0.16 |
| 6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | 0.003 | 0.01 | 0.043 | 0.161 |
| methyl 7-hydroxycoumarin-3-carboxylate | 0 | 0 | 1E-03 | 0 | 0 | 1E-03 | 0.002 | 0.004 | 0.01 | 0.033 | 0.137 |
| 3-acetyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | 0.001 | 0.001 | 0.001 | 0 | 0 | 0.004 | 0.011 | 0.055 | 0.158 |
| 6-ethyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.033 | -0.032 | -0.027 | -0.028 | -0.026 | -0.03 | -0.02 | 0.012 | 0.127 |
| 3-butyl-6-chloro-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | 0 | 0.001 | 0.003 | 0.005 | 0.008 | 0.018 | 0.069 | 0.173 |
| 7-hydroxycoumarin-3-carboxylic acid | 0 | 0 | 0.002 | 0.001 | 0.001 | 0.002 | 0.004 | 0.007 | 0.016 | 0.049 | 0.177 |
| 7-hydroxy-3-methoxyacetylcoumarin | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.001 | 0.003 | 0.007 | 0.014 | 0.051 | 0.158 |
| 8-acetyl-7-hydroxy-4-methylcoumarin | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0.001 | 0.004 | 0.01 | 0.05 | 0.149 |
| 7-hydroxy-4-methyl-3-propionic acid | 0 | 0 | 1E-03 | 1E-03 | 1E-03 | 1E-03 | 0.002 | 0.007 | 0.014 | 0.056 | 0.178 |
| Control (growth medium and solvent) | 0 | 0 | 0.002 | 0.001 | 0.002 | 0.002 | 0.005 | 0.007 | 0.015 | 0.067 | 0.174 |

## Appendix 4.3: Growth inhibition study over a wide concentration range of 7-hydroxy4-methylcoumarin (4-MU) with 8 strains of *E. coli*.

| ***E. coli* NCTC 10418** | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | 0 | 0 | 0 | 0.001 | 0.002 | 0.004 | 0.008 | 0.014 |
| 0.5 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.004 | 0.007 | 0.014 | 0.034 | 0.071 |
| 0.25 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.005 | 0.01 | 0.023 | 0.058 | 0.101 |
| 0.125 mmol $l^{-1}$ | 0 | -0.001 | 0 | 0.001 | 0.001 | 0.003 | 0.006 | 0.012 | 0.028 | 0.068 | 0.119 |
| 0.0625 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.006 | 0.013 | 0.031 | 0.072 | 0.13 |
| 0.03125 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | -0.001 | 0 | 0.001 | 0.005 | 0.013 | 0.033 | 0.08 | 0.135 |
| 0.015625 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.001 | 0.003 | 0.006 | 0.015 | 0.035 | 0.084 | 0.138 |
| 0.0078125 mmol $l^{-1}$ | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.007 | 0.017 | 0.039 | 0.093 | 0.152 |
| 0.00390625 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | 0 | 0 | 0.002 | 0.006 | 0.016 | 0.039 | 0.096 | 0.151 |
| 0.001953125 mmol $l^{-1}$ | 0 | 0 | 0 | 0 | 0 | 0.002 | 0.007 | 0.016 | 0.04 | 0.098 | 0.152 |
| Growth control | 0 | 0.001 | 0.001 | 0.002 | 0.003 | 0.005 | 0.009 | 0.019 | 0.043 | 0.103 | 0.156 |
| Broth control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| ***E. coli* FRHECO1** | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 mmol $l^{-1}$ | 0 | -0.003 | -0.002 | -0.002 | -0.001 | 0 | 0.002 | 0.006 | 0.015 | 0.037 | 0.073 |
| 0.5 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | 0 | 0 | 0.002 | 0.005 | 0.01 | 0.024 | 0.058 | 0.11 |
| 0.25 mmol $l^{-1}$ | 0 | -0.002 | -0.001 | -0.001 | 0 | 0.002 | 0.005 | 0.014 | 0.033 | 0.08 | 0.141 |
| 0.125 mmol $l^{-1}$ | 0 | -0.001 | 0 | 0 | 0.001 | 0.002 | 0.005 | 0.011 | 0.027 | 0.061 | 0.1 |
| 0.0625 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.001 | 0.003 | 0.008 | 0.018 | 0.041 | 0.096 | 0.157 |
| 0.03125 mmol $l^{-1}$ | 0 | -1E-03 | -1E-03 | 0 | 0.001 | 0.002 | 0.007 | 0.016 | 0.041 | 0.093 | 0.153 |
| 0.015625 mmol $l^{-1}$ | 0 | 0.003 | 0.004 | 0.005 | 0.005 | 0.008 | 0.015 | 0.03 | 0.071 | 0.143 | 0.226 |
| 0.0078125 mmol $l^{-1}$ | 0 | 0 | 0 | 0 | 0.001 | 0.003 | 0.009 | 0.022 | 0.056 | 0.113 | 0.184 |
| 0.00390625 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.002 | 0.005 | 0.012 | 0.028 | 0.072 | 0.141 | 0.229 |
| 0.001953125 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.002 | 0.004 | 0.011 | 0.027 | 0.072 | 0.139 | 0.229 |
| Growth control | 0 | 0.003 | 0.003 | 0.005 | 0.004 | 0.008 | 0.015 | 0.032 | 0.076 | 0.143 | 0.232 |
| Broth control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -0.001 | 0 | 0 |

| ***E. coli* FRHECO2** | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 mmol $l^{-1}$ | 0 | -0.002 | -0.002 | -0.001 | -0.001 | 0 | 0.002 | 0.006 | 0.015 | 0.035 | 0.065 |
| 0.5 mmol $l^{-1}$ | 0 | 0.001 | 0.001 | 0.002 | 0.002 | 0.003 | 0.005 | 0.01 | 0.022 | 0.048 | 0.083 |
| 0.25 mmol $l^{-1}$ | 0 | -0.002 | -0.001 | -0.001 | -0.001 | 0.001 | 0.004 | 0.01 | 0.026 | 0.062 | 0.107 |
| 0.125 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | -0.001 | 0 | 0.001 | 0.005 | 0.013 | 0.031 | 0.071 | 0.124 |
| 0.0625 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.002 | 0.004 | 0.009 | 0.02 | 0.042 | 0.095 | 0.156 |
| 0.03125 mmol $l^{-1}$ | 0 | 0 | -0.001 | -0.001 | -0.002 | 0.002 | 0.006 | 0.018 | 0.046 | 0.104 | 0.168 |
| 0.015625 mmol $l^{-1}$ | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.005 | 0.011 | 0.026 | 0.059 | 0.122 | 0.196 |
| 0.0078125 mmol $l^{-1}$ | 0 | -0.002 | -0.002 | -1E-03 | 0 | 0.003 | 0.011 | 0.028 | 0.074 | 0.143 | 0.226 |
| 0.00390625 mmol $l^{-1}$ | 0 | 0.001 | 0.001 | 0.002 | 0.003 | 0.006 | 0.014 | 0.033 | 0.081 | 0.149 | 0.237 |
| 0.001953125 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | 0 | 0.001 | 0.004 | 0.013 | 0.032 | 0.085 | 0.154 | 0.251 |
| Growth control | 0 | 0 | 0 | 0.001 | 0.002 | 0.006 | 0.014 | 0.033 | 0.084 | 0.15 | 0.247 |
| Broth control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Appendix 4.3 (Cont'd..): Growth inhibition study over a wide concentration range of 7-hydroxy4-methylcoumarin (4-MU) with 8 strains of *E. coli*.**

| ***E. coli* FRHECO3** | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 mmol $l^{-1}$ | 0 | -0.002 | -0.001 | -0.001 | -0.001 | 0 | 0.001 | 0.004 | 0.011 | 0.027 | 0.055 |
| 0.5 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.003 | 0.007 | 0.014 | 0.031 | 0.062 |
| 0.25 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | -0.001 | 0 | 0 | 0.002 | 0.006 | 0.016 | 0.037 | 0.072 |
| 0.125 mmol $l^{-1}$ | 0 | 0 | 0.001 | 0.002 | 0.001 | 0.003 | 0.006 | 0.013 | 0.027 | 0.059 | 0.11 |
| 0.0625 mmol $l^{-1}$ | 0 | 0.001 | 0 | 0.001 | 0.001 | 0.002 | 0.004 | 0.009 | 0.022 | 0.052 | 0.095 |
| 0.03125 mmol $l^{-1}$ | 0 | -0.002 | -0.002 | -0.001 | -0.001 | 0.001 | 0.004 | 0.013 | 0.033 | 0.078 | 0.139 |
| 0.015625 mmol $l^{-1}$ | 0 | 0.001 | 0.002 | 0.002 | 0.003 | 0.005 | 0.009 | 0.019 | 0.045 | 0.096 | 0.162 |
| 0.0078125 mmol $l^{-1}$ | 0 | -0.002 | -0.002 | -0.002 | -0.001 | 0.001 | 0.007 | 0.019 | 0.054 | 0.115 | 0.191 |
| 0.00390625 mmol $l^{-1}$ | 0 | 0.002 | 0.002 | 0.002 | 0.003 | 0.005 | 0.011 | 0.025 | 0.063 | 0.129 | 0.208 |
| 0.001953125 mmol $l^{-1}$ | 0 | 0 | 0 | 0 | 0.001 | 0.003 | 0.008 | 0.022 | 0.061 | 0.125 | 0.207 |
| Growth control | 0 | 0.001 | 0 | 0.001 | 0.002 | 0.004 | 0.01 | 0.025 | 0.063 | 0.128 | 0.21 |
| Broth control | 0 | 0 | -0.001 | 0 | 0 | -0.001 | -0.001 | -0.001 | -0.001 | 0 | -0.001 |

| ***E. coli* FRHECO4** | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 mmol $l^{-1}$ | 0 | -0.003 | -0.005 | -0.005 | -0.004 | -0.004 | -0.002 | -1E-03 | 0.004 | 0.013 | 0.029 |
| 0.5 mmol $l^{-1}$ | 0 | -0.002 | -0.002 | -0.002 | -0.002 | -0.001 | 0 | 0.003 | 0.008 | 0.021 | 0.04 |
| 0.25 mmol $l^{-1}$ | 0 | -0.002 | -0.002 | -0.003 | -0.002 | -0.002 | 0 | 0.003 | 0.011 | 0.025 | 0.045 |
| 0.125 mmol $l^{-1}$ | 0 | -0.001 | 0 | 0 | 0 | 0.001 | 0.003 | 0.008 | 0.017 | 0.034 | 0.056 |
| 0.0625 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | -0.002 | -0.001 | 0 | 0.004 | 0.01 | 0.026 | 0.057 | 0.08 |
| 0.03125 mmol $l^{-1}$ | 0 | -0.004 | -0.004 | -0.004 | -0.003 | -0.002 | 0 | 0.006 | 0.019 | 0.036 | 0.068 |
| 0.015625 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | 0 | 0 | 0.002 | 0.008 | 0.019 | 0.047 | 0.08 | 0.131 |
| 0.0078125 mmol $l^{-1}$ | 0 | -0.002 | -0.002 | -0.003 | -0.002 | -0.001 | 0.003 | 0.012 | 0.033 | 0.058 | 0.099 |
| 0.00390625 mmol $l^{-1}$ | 0 | 0 | -0.001 | 0 | 0.001 | 0.004 | 0.009 | 0.024 | 0.062 | 0.097 | 0.157 |
| 0.001953125 mmol $l^{-1}$ | 0 | -0.002 | -0.002 | -0.002 | -0.002 | 0.001 | 0.007 | 0.022 | 0.06 | 0.094 | 0.156 |
| Growth control | 0 | 0.001 | 0 | 0 | 0.001 | 0.004 | 0.009 | 0.024 | 0.063 | 0.095 | 0.156 |
| Broth control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -0.001 | -0.001 | -0.001 |

| ***E. coli* FRHECO5** | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | -0.001 | 0 | 0 | 0.001 | 0.002 | 0.004 | 0.009 | 0.018 |
| 0.5 mmol $l^{-1}$ | 0 | 0.001 | 0.001 | 0.001 | 0 | 0.002 | 0.005 | 0.004 | 0.007 | 0.013 | 0.023 |
| 0.25 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | 0 | 0 | 0 | 0.001 | 0.003 | 0.006 | 0.013 | 0.027 |
| 0.125 mmol $l^{-1}$ | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.003 | 0.007 | 0.015 | 0.034 |
| 0.0625 mmol $l^{-1}$ | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.004 | 0.009 | 0.019 | 0.045 |
| 0.03125 mmol $l^{-1}$ | 0 | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.004 | 0.01 | 0.024 | 0.059 |
| 0.015625 mmol $l^{-1}$ | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.002 | 0.004 | 0.007 | 0.014 | 0.034 | 0.076 |
| 0.0078125 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | 0 | 0 | 0.001 | 0.003 | 0.007 | 0.018 | 0.046 | 0.1 |
| 0.00390625 mmol $l^{-1}$ | 0 | 0 | 0 | 0 | 0.001 | 0.002 | 0.003 | 0.008 | 0.02 | 0.053 | 0.109 |
| 0.001953125 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | 0 | 0 | 0 | 0.003 | 0.008 | 0.021 | 0.057 | 0.117 |
| Growth control | 0 | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.004 | 0.009 | 0.023 | 0.058 | 0.117 |
| Broth control | 0 | 0.002 | 0.003 | 0.002 | 0.003 | 0.002 | 0.003 | 0.003 | 0.002 | 0.003 | 0.003 |

**Appendix 4.3 (Cont'd..): Growth inhibition study over a wide concentration range of 7-hydroxy4-methylcoumarin (4-MU) with 8 strains of *E. coli*.**

| ***E. coli* FRHECO6** | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 mmol $l^{-1}$ | 0 | 0.003 | 0 | 0 | 0 | 0.006 | 0.005 | 0.01 | 0.021 | 0.044 | 0.066 |
| 0.5 mmol $l^{-1}$ | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.003 | 0.006 | 0.013 | 0.027 | 0.056 | 0.084 |
| 0.25 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.001 | 0.003 | 0.006 | 0.013 | 0.028 | 0.055 | 0.086 |
| 0.125 mmol $l^{-1}$ | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.004 | 0.007 | 0.016 | 0.036 | 0.069 | 0.106 |
| 0.0625 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.001 | 0.003 | 0.008 | 0.018 | 0.045 | 0.083 | 0.132 |
| 0.03125 mmol $l^{-1}$ | 0 | -0.001 | 0.001 | 0.001 | 0.002 | 0.004 | 0.011 | 0.023 | 0.057 | 0.113 | 0.181 |
| 0.015625 mmol $l^{-1}$ | 0 | 0.001 | 0.002 | 0.003 | 0.003 | 0.006 | 0.014 | 0.03 | 0.074 | 0.13 | 0.216 |
| 0.0078125 mmol $l^{-1}$ | 0 | -0.001 | 0 | 0 | 0.001 | 0.004 | 0.013 | 0.033 | 0.08 | 0.147 | 0.252 |
| 0.00390625 mmol $l^{-1}$ | 0 | 0 | 0 | 0.001 | 0.002 | 0.006 | 0.017 | 0.042 | 0.098 | 0.176 | 0.304 |
| 0.001953125 mmol $l^{-1}$ | 0 | -0.001 | -0.001 | 0 | 0.001 | 0.005 | 0.017 | 0.045 | 0.103 | 0.184 | 0.32 |
| Growth control | 0 | -0.001 | -0.001 | 0 | 0.002 | 0.007 | 0.018 | 0.048 | 0.108 | 0.188 | 0.328 |
| Broth control | 0 | -0.001 | -0.001 | -0.001 | 0 | 0 | 0 | -0.001 | -0.001 | 0 | -0.001 |

| ***E. coli* FRHECO7** | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **210** | **240** | **270** | **300** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 mmol $l^{-1}$ | 0 | -0.001 | 0.001 | 0.001 | 0.002 | 0.002 | 0.006 | 0.007 | 0.015 | 0.029 | 0.043 |
| 0.5 mmol $l^{-1}$ | 0 | 0.002 | 0.002 | 0.002 | 0.002 | 0.004 | 0.006 | 0.01 | 0.021 | 0.042 | 0.061 |
| 0.25 mmol $l^{-1}$ | 0 | 0 | 0.001 | 0.001 | 0.001 | 0.003 | 0.005 | 0.01 | 0.022 | 0.045 | 0.072 |
| 0.125 mmol $l^{-1}$ | 0 | 0 | 0 | 0 | 0 | 0.002 | 0.004 | 0.01 | 0.024 | 0.051 | 0.084 |
| 0.0625 mmol $l^{-1}$ | 0 | 0.002 | 0.007 | 0.009 | 0.009 | 0.01 | 0.01 | 0.02 | 0.037 | 0.066 | 0.114 |
| 0.03125 mmol $l^{-1}$ | 0 | 0.001 | 0.001 | 0.001 | 0.002 | 0.003 | 0.006 | 0.013 | 0.031 | 0.061 | 0.101 |
| 0.015625 mmol $l^{-1}$ | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.004 | 0.008 | 0.017 | 0.042 | 0.082 | 0.141 |
| 0.0078125 mmol $l^{-1}$ | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.004 | 0.009 | 0.021 | 0.053 | 0.103 | 0.175 |
| 0.00390625 mmol $l^{-1}$ | 0 | 0.001 | 0.001 | 0.001 | 0.002 | 0.005 | 0.011 | 0.025 | 0.064 | 0.119 | 0.201 |
| 0.001953125 mmol $l^{-1}$ | 0 | 0.001 | 0.001 | 0.002 | 0.003 | 0.005 | 0.012 | 0.029 | 0.067 | 0.128 | 0.222 |
| Growth control | 0 | 0 | 0 | 0 | 0.002 | 0.004 | 0.011 | 0.028 | 0.066 | 0.129 | 0.221 |
| Broth control | 0 | 0.001 | 0.001 | 0 | 0.001 | 0 | 0.001 | 0 | 0 | 0 | 0 |

## Appendix 4.4: Absorbance (690 nm) increase over 18 h incubation at 37oC in the presence of 6 ß-D-galactoside substrates with 12 coliform organisms.

| ***E. coli* NCTC 10418** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.001 | 0.002 | 0.021 | 0.055 | 0.148 | 0.206 | 0.084 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.000 | 0.002 | 0.021 | 0.071 | 0.171 | 0.130 | 0.115 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -0.001 | 0.002 | 0.018 | 0.051 | 0.105 | 0.144 | 0.150 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.000 | 0.003 | 0.029 | 0.095 | 0.163 | 0.155 | 0.207 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.131 | 0.092 | 0.081 | 0.188 | 0.072 | 0.243 | 0.122 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.022 | 0.020 | 0.071 | 0.079 | 0.136 | 0.204 | 0.076 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0.001 | 0.03 | 0.06 | 0.098 | 0.14 | 0.161 |

| ***E. coli* FRHECO1** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.001 | 0.003 | 0.018 | 0.090 | 0.200 | 0.229 | 0.400 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.000 | 0.002 | 0.022 | 0.099 | 0.113 | 0.243 | 0.356 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -0.001 | 0.001 | 0.023 | 0.079 | 0.177 | 0.245 | 0.366 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.003 | 0.005 | 0.019 | 0.055 | 0.176 | 0.226 | 0.299 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.133 | 0.100 | 0.100 | 0.196 | 0.200 | 0.234 | 0.255 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.002 | 0.002 | 0.035 | 0.064 | 0.149 | 0.228 | 0.284 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0.029 | 0.102 | 0.199 | 0.216 | 0.299 |

| ***E. coli* FRHECO2** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.001 | 0.003 | 0.018 | 0.085 | 0.191 | 0.230 | 0.376 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.002 | 0.003 | 0.018 | 0.086 | 0.184 | 0.222 | 0.356 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -0.001 | 0.002 | 0.018 | 0.086 | 0.185 | 0.223 | 0.393 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.003 | 0.006 | 0.023 | 0.098 | 0.194 | 0.226 | 0.329 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.160 | 0.242 | 0.100 | 0.245 | 0.269 | 0.254 | 0.255 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.002 | 0.003 | 0.024 | 0.099 | 0.204 | 0.239 | 0.271 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0.003 | 0.01 | 0.049 | 0.172 | 0.219 | 0.238 | 0.397 |

| ***E. coli* FRHECO3** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.001 | 0.001 | 0.005 | 0.031 | 0.114 | 0.214 | 0.379 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.000 | 0.001 | 0.005 | 0.030 | 0.114 | 0.216 | 0.385 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | 0.002 | 0.001 | 0.006 | 0.033 | 0.115 | 0.223 | 0.393 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.001 | 0.001 | 0.006 | 0.035 | 0.131 | 0.257 | 0.371 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.263 | 0.184 | 0.167 | 0.261 | 0.230 | 0.341 | 0.298 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.005 | 0.005 | 0.009 | 0.039 | 0.134 | 0.242 | 0.382 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0.003 | 0.014 | 0.07 | 0.216 | 0.287 | 0.297 | 0.495 |

| ***K. pneumoniae* NCTC 10896** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -0.001 | 0.001 | -0.001 | -0.001 | 0.000 | 0.004 | 0.007 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.000 | 0.000 | -0.001 | 0.002 | 0.001 | 0.000 | 0.004 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | 0.000 | 0.001 | 0.000 | 0.003 | 0.002 | 0.003 | 0.006 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.001 | 0.000 | 0.000 | -0.001 | 0.001 | 0.002 | 0.005 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.177 | 0.152 | 0.139 | 0.207 | 0.110 | 0.172 | 0.315 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.003 | 0.001 | 0.001 | -0.001 | 0.001 | -0.001 | 0.050 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0.001 | 0.001 | 0.002 | 0.003 | 0.002 | 0.019 |

| ***K. pneumoniae* FRHKPN1** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.002 | 0.007 | 0.047 | 0.181 | 0.275 | 0.333 | 0.432 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.002 | 0.006 | 0.047 | 0.183 | 0.276 | 0.336 | 0.427 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | 0.001 | 0.007 | 0.047 | 0.185 | 0.279 | 0.342 | 0.498 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.001 | 0.007 | 0.050 | 0.193 | 0.280 | 0.337 | 0.418 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.287 | 0.263 | 0.260 | 0.511 | 0.607 | 0.471 | 0.484 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.006 | 0.008 | 0.049 | 0.178 | 0.272 | 0.319 | 0.338 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0.002 | 0.01 | 0.047 | 0.186 | 0.224 | 0.263 | 0.439 |

**Appendix 4.4 (Cont'd..): Absorbance (690 nm) increase over 18 h incubation at 37oC in the presence of 6 ß-D-galactoside substrates with 12 coliform organisms.**

| ***K. pneumoniae* FRHKPN2** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.002 | 0.007 | 0.050 | 0.188 | 0.289 | 0.345 | 0.428 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.009 | 0.008 | 0.049 | 0.193 | 0.287 | 0.353 | 0.437 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | 0.001 | 0.007 | 0.050 | 0.191 | 0.286 | 0.370 | 0.443 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.001 | 0.009 | 0.053 | 0.195 | 0.285 | 0.368 | 0.410 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.231 | 0.251 | 0.308 | 0.457 | 0.556 | 0.492 | 0.491 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.003 | 0.009 | 0.052 | 0.188 | 0.286 | 0.328 | 0.409 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0.003 | 0.015 | 0.08 | 0.204 | 0.245 | 0.285 | 0.475 |

| ***K. pneumoniae* FRHKPN3** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.002 | 0.008 | 0.050 | 0.185 | 0.288 | 0.344 | 0.420 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.007 | 0.008 | 0.044 | 0.185 | 0.269 | 0.369 | 0.444 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | 0.000 | 0.006 | 0.050 | 0.190 | 0.280 | 0.340 | 0.400 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.001 | 0.009 | 0.052 | 0.194 | 0.284 | 0.366 | 0.408 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.222 | 0.260 | 0.311 | 0.449 | 0.523 | 0.488 | 0.485 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.002 | 0.010 | 0.055 | 0.165 | 0.228 | 0.312 | 0.404 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0.003 | 0.012 | 0.082 | 0.222 | 0.262 | 0.288 | 0.464 |

| ***C. freundii* NCTC 9750** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.001 | 0.003 | 0.014 | 0.052 | 0.169 | 0.787 | 0.704 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.001 | 0.002 | 0.014 | 0.054 | 0.160 | 0.764 | 0.695 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | 0.000 | 0.003 | 0.014 | 0.054 | 0.142 | 0.656 | 0.746 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.001 | 0.003 | 0.014 | 0.053 | 0.153 | 0.704 | 0.704 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.282 | 0.335 | 0.249 | 0.388 | 0.348 | 0.794 | 0.578 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.003 | 0.003 | 0.012 | 0.046 | 0.123 | 0.630 | 0.612 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0.001 | 0.003 | 0.015 | 0.049 | 0.155 | 0.689 | 0.700 |

| ***C. freundii* FRHCFR1** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.002 | 0.004 | 0.017 | 0.073 | 0.266 | 0.664 | 0.338 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.001 | 0.004 | 0.018 | 0.074 | 0.206 | 0.667 | 0.252 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | 0.001 | 0.004 | 0.015 | 0.067 | 0.198 | 0.564 | 0.341 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.004 | 0.004 | 0.019 | 0.073 | 0.224 | 0.613 | 0.349 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.374 | 0.408 | 0.486 | 0.454 | 0.447 | 0.456 | 0.157 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.002 | 0.003 | 0.013 | 0.058 | 0.205 | 0.641 | 0.295 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0.003 | 0.007 | 0.037 | 0.287 | 0.582 | 0.7 | 1.168 |

| ***C. freundii* FRHCFR2** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.001 | 0.005 | 0.020 | 0.241 | 0.185 | 0.451 | 0.310 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.000 | 0.004 | 0.019 | 0.188 | 0.192 | 0.464 | 0.387 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | 0.000 | 0.006 | 0.019 | 0.205 | 0.177 | 0.454 | 0.419 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.001 | 0.004 | 0.019 | 0.231 | 0.168 | 0.405 | 0.318 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.369 | 0.362 | 0.358 | 0.466 | 0.205 | 0.466 | 0.315 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.001 | 0.001 | 0.012 | 0.062 | 0.222 | 0.317 | 0.471 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0.003 | 0.007 | 0.041 | 0.187 | 0.151 | 0.474 | 0.791 |

| ***C. freundii* FRHCFR3** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | 0.002 | 0.003 | 0.011 | 0.199 | 0.205 | 0.461 | 0.268 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | 0.001 | 0.004 | 0.019 | 0.176 | 0.199 | 0.502 | 0.444 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | 0.001 | 0.003 | 0.025 | 0.098 | 0.200 | 0.488 | 0.518 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0.004 | 0.003 | 0.015 | 0.184 | 0.235 | 0.526 | 0.318 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.374 | 0.388 | 0.389 | 0.420 | 0.444 | 0.488 | 0.316 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0.002 | 0.001 | 0.011 | 0.099 | 0.201 | 0.365 | 0.471 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0.003 | 0.007 | 0.033 | 0.126 | 0.268 | 0.399 | 0.650 |

## Appendix 4.5: Fluorescence (365/440 nm) increase over 18 h incubation at 37oC in the presence of 6 ß-D-galactoside substrates with 12 coliform organisms.

| ***E. coli* NCTC 10418** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -246 | -267 | -228 | -249 | -152 | -255 | -310 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -307 | -323 | -299 | -310 | -43 | 523 | 24351 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -249 | -284 | -243 | -245 | 26 | 726 | 40662 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -312 | -358 | -300 | -28 | 3769 | 16146 | 28508 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -236 | -250 | -202 | -120 | 608 | 3611 | 31664 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -214 | -221 | -175 | -85 | 822 | 3588 | 21780 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -288 | -333 | -288 | -64 | 2041 | 11023 | 26412 |

| ***E. coli* FRHECO1** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -240 | -264 | -229 | -251 | -129 | -240 | -213 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -304 | -330 | -279 | -306 | -200 | -151 | 18032 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -254 | -275 | -242 | -239 | -101 | 102 | 38406 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -325 | -359 | -268 | -292 | 959 | 14348 | 32088 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -204 | -226 | -188 | -170 | 51 | 989 | 35303 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -203 | -217 | -172 | -150 | 533 | 3356 | 28944 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -360 | -344 | -365 | -133 | 8825 | 18889 | 26314 |

| ***E. coli* FRHECO2** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -260 | -275 | -244 | -248 | -165 | -245 | -174 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -287 | -326 | -281 | -307 | -199 | -188 | 11183 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -262 | -275 | -251 | -255 | -147 | -64 | 29641 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -293 | -321 | -227 | -258 | 235 | 3666 | 34636 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -219 | -233 | -197 | -193 | -45 | 381 | 37126 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -209 | -211 | -168 | -170 | 37 | 873 | 25398 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -397 | -388 | -404 | 21 | 7965 | 15573 | 26161 |

| ***E. coli* FRHECO3** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -247 | -262 | -231 | -235 | -152 | -232 | -161 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -274 | -313 | -268 | -294 | -186 | -175 | 11196 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -249 | -262 | -238 | -242 | -134 | -51 | 29654 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -280 | -308 | -214 | -245 | 248 | 3679 | 34649 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -206 | -220 | -184 | -180 | -32 | 394 | 37139 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -196 | -198 | -155 | -157 | 50 | 886 | 25411 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -384 | -375 | -391 | 34 | 7978 | 15586 | 26174 |

| ***K. pneumoniae* NCTC 10896** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -266 | -283 | -243 | -266 | -197 | -308 | -258 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -307 | -318 | -270 | -297 | -205 | -318 | 15 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -242 | -248 | -199 | -217 | -132 | -234 | 113 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -247 | -161 | 55 | 133 | 426 | 308 | 2687 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -200 | -196 | -124 | -116 | -14 | -78 | 487 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -181 | -151 | -52 | -32 | 95 | 18 | 8265 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -236 | -199 | -26 | 35 | 109 | 289 | 2025 |

**Appendix 4.5 (Cont'd..): Fluorescence (365/440 nm) increase over 18 h incubation at 37oC in the presence of 6 ß-D-galactoside substrates with 12 coliform organisms.**

| ***K. pneumoniae* FRHKPN1** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -246 | -279 | -228 | -225 | -140 | -225 | -158 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -306 | -327 | -284 | -289 | -158 | -153 | 16876 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -267 | -267 | -241 | -209 | 165 | 594 | 35883 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -385 | -414 | -348 | -322 | 661 | 4391 | 35726 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -221 | -235 | -193 | -188 | 26 | 151 | 36986 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -206 | -235 | -170 | -87 | 1509 | 3652 | 34807 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -384 | -366 | -414 | -305 | 1523 | 8668 | 25878 |

| ***K. pneumoniae* FRHKPN2** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -247 | -255 | -217 | -227 | -131 | -224 | -145 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -324 | -345 | -305 | -322 | -171 | -167 | 11270 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -268 | -272 | -246 | -226 | 203 | 691 | 27709 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -354 | -408 | -323 | -250 | 852 | 5288 | 36098 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -221 | -232 | -193 | -194 | 64 | 267 | 33174 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -224 | -237 | -186 | -57 | 1795 | 3944 | 28800 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -402 | -384 | -423 | -357 | 294 | 2680 | 27209 |

| ***K. pneumoniae* FRHKPN3** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -236 | -243 | -205 | -216 | -120 | -213 | -133 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -313 | -334 | -294 | -311 | -160 | -155 | 11281 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -256 | -261 | -235 | -215 | 214 | 703 | 27721 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -343 | -396 | -312 | -239 | 863 | 5299 | 36110 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -210 | -221 | -181 | -182 | 76 | 279 | 33185 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -213 | -225 | -175 | -45 | 1807 | 3955 | 28812 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -391 | -373 | -411 | -346 | 305 | 2692 | 27221 |

| ***C. freundii* NCTC 9750** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -246 | -271 | -217 | -253 | -150 | -199 | -133 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -330 | -345 | -308 | -325 | -145 | 420 | 13509 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -256 | -277 | -243 | -257 | -34 | 784 | 34211 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -345 | -386 | -316 | -286 | 1713 | 8975 | 36217 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -230 | -235 | -212 | -214 | 232 | 1775 | 38917 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -229 | -230 | -188 | -194 | 553 | 1980 | 30194 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -265 | -235 | -216 | -188 | 642 | 2156 | 35987 |

| ***C. freundii* FRHCFR1** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -272 | -293 | -252 | -262 | -151 | -232 | -177 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -313 | -332 | -286 | -303 | -145 | 795 | 12005 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -270 | -292 | -249 | -261 | -57 | 1334 | 30523 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -341 | -376 | -274 | -239 | 1064 | 13781 | 35803 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -211 | -209 | -179 | -174 | 98 | 3284 | 39513 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -203 | -219 | -181 | -171 | 313 | 2974 | 27528 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -385 | -372 | -406 | 302 | 8673 | 21827 | 26289 |

**Appendix 4.5 (Cont'd..): Fluorescence (365/440 nm) increase over 18 h incubation at 37oC in the presence of 6 ß-D-galactoside substrates with 12 coliform organisms.**

| ***C. freundii* FRHCFR2** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -257 | -281 | -220 | -222 | -147 | -222 | -163 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -323 | -340 | -293 | -215 | 921 | 1942 | 12978 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -263 | -281 | -249 | -120 | 1789 | 3791 | 31895 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -368 | -389 | -293 | 604 | 14258 | 25371 | 35986 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -205 | -216 | -158 | 9 | 4700 | 10025 | 39751 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -205 | -231 | -177 | 64 | 3270 | 5140 | 26437 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -443 | -402 | -458 | -168 | 4843 | 14552 | 26110 |

| ***C. freundii* FRHCFR3** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| Growth control | 0 | -247 | -271 | -210 | -212 | -137 | -212 | -153 |
| 7-hydroxy-4-methyl-3-propionic acid-ß-D-galactoside | 0 | -313 | -330 | -283 | -205 | 930 | 1951 | 12987 |
| 7-hydroxycoumarin-4-acetic acid-ß-D-galactoside | 0 | -253 | -271 | -239 | -110 | 1798 | 3800 | 31905 |
| ethyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -358 | -379 | -283 | 614 | 14267 | 25381 | 35996 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -195 | -206 | -148 | 19 | 4710 | 10034 | 39760 |
| 7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -195 | -221 | -167 | 74 | 3280 | 5149 | 26446 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -433 | -392 | -448 | -158 | 4853 | 14562 | 26119 |

## Appendix 4.6: Absorbance (690 nm) increase over 18 h incubation at 37oC in the presence of 5 ß-D-glucuronide substrates with 6 *E. coli* strains.

| ***E. coli* NCTC 10418** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.000 | -0.001 | -0.001 | 0.003 | -0.001 | -0.001 | 0.042 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.000 | 0.000 | 0.000 | 0.000 | -0.001 | -0.001 | 0.053 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -0.002 | -0.001 | 0.002 | -0.004 | -0.005 | -0.007 | 0.069 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -0.001 | -0.002 | -0.002 | -0.004 | -0.003 | -0.002 | 0.118 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | -0.002 | -0.003 | -0.004 | 0.001 | 0.001 | -0.005 | 0.092 |
| Growth control | 0 | -0.002 | -0.003 | -0.004 | -0.005 | -0.005 | -0.005 | 0.047 |

| ***E. coli* FRHECO2** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.005 | 0.011 | 0.011 | 0.142 | 0.220 | 0.263 | 0.383 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.003 | 0.007 | 0.007 | 0.140 | 0.225 | 0.256 | 0.377 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.000 | 0.001 | 0.001 | -0.001 | 0.002 | 0.007 | 0.368 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | 0.002 | 0.011 | 0.043 | 0.122 | 0.195 | 0.214 | 0.363 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | 0.001 | 0.009 | 0.043 | 0.125 | 0.204 | 0.213 | 0.358 |
| Growth control | 0 | 0.000 | 0.008 | 0.045 | 0.129 | 0.200 | 0.223 | 0.364 |

| ***E. coli* FRHECO4** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.005 | 0.011 | 0.011 | 0.172 | 0.253 | 0.314 | 0.417 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.004 | 0.009 | 0.009 | 0.174 | 0.288 | 0.344 | 0.422 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.001 | 0.004 | 0.012 | 0.037 | 0.143 | 0.240 | 0.425 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -0.001 | 0.010 | 0.062 | 0.178 | 0.273 | 0.336 | 0.429 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | 0.001 | 0.010 | 0.057 | 0.172 | 0.264 | 0.324 | 0.383 |
| Growth control | 0 | 0.001 | 0.011 | 0.059 | 0.173 | 0.265 | 0.328 | 0.457 |

| ***E. coli* FRHECO5** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.003 | 0.006 | 0.006 | 0.120 | 0.260 | 0.273 | 0.489 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.002 | 0.004 | 0.004 | 0.136 | 0.231 | 0.290 | 0.501 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.002 | 0.005 | 0.015 | 0.051 | 0.143 | 0.258 | 0.516 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | 0.003 | 0.012 | 0.057 | 0.138 | 0.256 | 0.336 | 0.511 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | 0.001 | 0.009 | 0.050 | 0.134 | 0.240 | 0.319 | 0.477 |
| Growth control | 0 | 0.000 | 0.009 | 0.050 | 0.140 | 0.292 | 0.317 | 0.507 |

| ***E. coli* FRHECO6** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.002 | 0.005 | 0.005 | 0.099 | 0.199 | 0.250 | 0.446 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.003 | 0.005 | 0.005 | 0.112 | 0.219 | 0.273 | 0.468 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -0.001 | 0.002 | 0.010 | 0.009 | 0.022 | 0.059 | 0.447 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -0.001 | 0.007 | 0.044 | 0.126 | 0.277 | 0.288 | 0.459 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | 0.006 | 0.010 | 0.044 | 0.125 | 0.283 | 0.289 | 0.421 |
| Growth control | 0 | 0.001 | 0.010 | 0.046 | 0.127 | 0.292 | 0.290 | 0.428 |

| ***E. coli* FRHECO8** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.006 | 0.011 | 0.011 | 0.165 | 0.259 | 0.316 | 0.427 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.005 | 0.010 | 0.010 | 0.175 | 0.264 | 0.322 | 0.449 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.001 | 0.002 | 0.004 | 0.007 | 0.056 | 0.168 | 0.431 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | 0.002 | 0.019 | 0.097 | 0.256 | 0.351 | 0.405 | 0.439 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | 0.002 | 0.018 | 0.094 | 0.247 | 0.349 | 0.400 | 0.436 |
| Growth control | 0 | 0.002 | 0.016 | 0.091 | 0.242 | 0.336 | 0.388 | 0.439 |

| **Control (organism free)** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.002 | 0.004 | 0.004 | 0.004 | 0.001 | 0.000 | 0.013 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.001 | 0.002 | 0.002 | 0.001 | 0.001 | -0.001 | 0.010 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | 0.001 | -0.001 | -0.001 | 0.000 | 0.000 | -0.001 | 0.004 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -0.001 | -0.001 | -0.001 | -0.002 | -0.001 | -0.001 | 0.001 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | 0.000 | -0.001 | -0.001 | 0.000 | -0.001 | -0.001 | 0.016 |
| Growth control | 0 | -0.001 | -0.001 | -0.002 | -0.002 | -0.001 | -0.001 | 0.020 |

## Appendix 4.7: Fluorescence (365/440 nm) increase over 18 h incubation at 37oC in the presence of 5 ß-D-glucuronide substrates with 6 *E. coli* strains.

| ***E. coli* NCTC 10418** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -473 | -591 | -539 | -638 | -1071 | -802 | -1613 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -246 | -244 | -164 | -167 | -394 | -142 | 246 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -199 | -252 | -307 | -332 | -394 | -385 | -654 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -400 | -531 | -684 | -739 | -919 | -922 | 22187 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | -166 | -191 | -223 | -238 | -261 | -262 | 25369 |
| Growth control | 0 | -164 | -195 | -231 | -242 | -287 | -269 | -219 |

| ***E. coli* FRHECO2** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -467 | -566 | -478 | -469 | 3254 | 13510 | 20442 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -195 | -87 | 100 | 650 | 23420 | 31515 | 34104 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -141 | -14 | 55 | 174 | 296 | 478 | 33794 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -473 | -539 | -624 | -489 | 16696 | 16869 | 30415 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | -151 | -157 | -188 | -104 | 5427 | 5397 | 27681 |
| Growth control | 0 | -173 | -202 | -217 | -207 | -216 | -182 | 89 |

| ***E. coli* FRHECO4** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -479 | -603 | -536 | -566 | -939 | -517 | 991 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -281 | -272 | -180 | -156 | -319 | 10 | 26979 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -209 | -255 | -315 | -304 | -315 | -205 | 27698 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -444 | -511 | -613 | -736 | -565 | 1757 | 30685 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | -160 | -188 | -219 | -197 | -155 | 123 | 28320 |
| Growth control | 0 | -176 | -204 | -231 | -202 | -204 | -153 | 113 |

| ***E. coli* FRHECO5** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -490 | -598 | -557 | -603 | -1006 | -562 | 1748 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -266 | -267 | -205 | -185 | -353 | -19 | 29031 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -231 | -286 | -335 | -324 | -342 | -226 | 30185 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -493 | -579 | -669 | -757 | -568 | 2077 | 32300 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | -163 | -193 | -243 | -203 | -157 | 435 | 30644 |
| Growth control | 0 | -167 | -198 | -226 | -219 | -211 | -163 | 179 |

| ***E. coli* FRHECO6** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -487 | -594 | -539 | -585 | -1005 | -594 | 4706 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -244 | -249 | -180 | -153 | -308 | 199 | 30394 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -203 | -257 | -324 | -303 | -350 | -270 | 30458 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -528 | -592 | -655 | -726 | 993 | 13974 | 31613 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | -154 | -194 | -221 | -200 | 155 | 3152 | 33936 |
| Growth control | 0 | -159 | -196 | -222 | -208 | -210 | -156 | 121 |

| ***E. coli* FRHECO8** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -478 | -590 | -539 | -574 | -971 | -418 | 3247 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -275 | -283 | -193 | -150 | -335 | 64 | 30125 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -243 | -270 | -342 | -347 | -366 | -281 | 30816 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -604 | -690 | -755 | -871 | 3997 | 22059 | 32654 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | -153 | -185 | -211 | -185 | 695 | 8015 | 33617 |
| Growth control | 0 | -170 | -208 | -223 | -187 | -208 | -159 | 139 |

| **Control (organism free)** | **0** | **60** | **120** | **180** | **240** | **300** | **360** | **18h** |
|---|---|---|---|---|---|---|---|---|
| ethyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -453 | -597 | -537 | -637 | -1113 | -864 | -1780 |
| benzyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -256 | -240 | -171 | -190 | -442 | -211 | -170 |
| methyl 7-hydroxycoumarin-3-carboxylate-glucuronide | 0 | -200 | -251 | -304 | -312 | -365 | -312 | 172 |
| 6-chloro-7-hydroxy-4-methylcoumarin-glucuronide | 0 | -539 | -620 | -726 | -725 | -797 | -720 | -719 |
| 7-hydroxy-4-methylcoumarin-glucuronide | 0 | -158 | -199 | -232 | -217 | -254 | -216 | 1318 |
| Growth control | 0 | -180 | -217 | -244 | -246 | -264 | -232 | 93 |

## Appendix 5.1: Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

**E. coli FRHECO2**

| | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-3}$ 4-MU-GAL | 0 | -427 | -448 | -354 | -430 | -451 | -186 | -381 | -445 | -378 | -195 | -439 | -439 | -421 | -189 | -226 | -201 | -67 |
| $10^{-3}$ 4-MU-GAL | 0 | -418 | -412 | -351 | -409 | -409 | -171 | -341 | -427 | -375 | -152 | -430 | -399 | -384 | -131 | -152 | -167 | -6 |
| $10^{-3}$ 4-MU-GAL | 0 | -86 | -138 | -132 | -138 | -135 | -168 | -49 | -141 | -104 | -162 | -196 | -162 | -150 | -254 | -196 | -217 | -58 |
| $10^{-3}$ 4-MU-GAL | 0 | -119 | -150 | -162 | -134 | -137 | -165 | -58 | -144 | -79 | -159 | -180 | -128 | -128 | -208 | -171 | -174 | 37 |
| $10^{-3}$ EHC-GAL | 0 | -721 | -717 | -583 | -745 | -760 | -217 | -638 | -791 | -675 | -257 | -782 | -766 | -739 | -244 | -287 | -290 | -235 |
| $10^{-3}$ EHC-GAL | 0 | -715 | -718 | -580 | -733 | -751 | -223 | -635 | -770 | -693 | -217 | -776 | -745 | -757 | -263 | -321 | -287 | -239 |
| $10^{-3}$ EHC-GAL | 0 | -52 | -128 | -143 | -125 | -101 | -177 | 76 | -140 | -40 | -143 | -214 | -125 | -125 | -302 | -232 | -241 | -3 |
| $10^{-3}$ EHC-GAL | 0 | -80 | -159 | -193 | -177 | -165 | -211 | 0 | -187 | -89 | -232 | -284 | -205 | -199 | -397 | -300 | -315 | -98 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -583 | -607 | -498 | -607 | -604 | -198 | -513 | -638 | -528 | -195 | -641 | -595 | -604 | -202 | -263 | -241 | -183 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -589 | -601 | -501 | -589 | -623 | -196 | -510 | -629 | -543 | -205 | -626 | -629 | -598 | -244 | -269 | -247 | -183 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -65 | -135 | -150 | -144 | -138 | -150 | 6 | -159 | -80 | -171 | -211 | -150 | -132 | -287 | -235 | -229 | -68 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -83 | -156 | -165 | -150 | -150 | -192 | -12 | -168 | -86 | -156 | -235 | -162 | -156 | -287 | -232 | -260 | -46 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -759 | -772 | -616 | -756 | -784 | -213 | -650 | -817 | -686 | -238 | -814 | -808 | -784 | -262 | -277 | -302 | -225 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -763 | -769 | -632 | -766 | -794 | -226 | -672 | -849 | -724 | -241 | -837 | -812 | -791 | -275 | -339 | -336 | -245 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -67 | -143 | -174 | -168 | -131 | -201 | 46 | -174 | -70 | -210 | -250 | -192 | -171 | -366 | -287 | -293 | -64 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -43 | -140 | -174 | -165 | -140 | -220 | 37 | -183 | -58 | -192 | -281 | -186 | -168 | -372 | -287 | -284 | -43 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1615 | -1615 | -1356 | -1578 | -1652 | -696 | -1392 | -1633 | -1423 | -654 | -1572 | -1529 | -1496 | -605 | -693 | -657 | -321 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1571 | -1584 | -1324 | -1553 | -1605 | -674 | -1370 | -1602 | -1404 | -580 | -1516 | -1504 | -1465 | -558 | -613 | -604 | -283 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -363 | -498 | -534 | -479 | -470 | -550 | -150 | -446 | -309 | -522 | -608 | -483 | -473 | -769 | -663 | -678 | -107 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -378 | -506 | -525 | -463 | -424 | -512 | -122 | -436 | -268 | -457 | -555 | -393 | -405 | -692 | -518 | -555 | 58 |
| $10^{-3}$ control | 0 | -354 | -354 | -283 | -335 | -341 | -115 | -286 | -357 | -286 | -109 | -335 | -305 | -323 | -100 | -112 | -100 | 46 |
| $10^{-3}$ control | 0 | -332 | -329 | -292 | -329 | -329 | -100 | -265 | -344 | -283 | -85 | -326 | -286 | -305 | -94 | -109 | -103 | 89 |
| $10^{-3}$ control | 0 | -67 | -73 | -113 | -86 | -76 | -101 | 33 | -70 | -18 | -83 | -95 | -52 | -61 | -128 | -95 | -104 | 101 |
| $10^{-3}$ control | 0 | -76 | -85 | -101 | -82 | -58 | -88 | 6 | -98 | -24 | -64 | -110 | -61 | -55 | -149 | -101 | -104 | 113 |

## Appendix 5.1: Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| E. coli FRHECO2 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-2}$ 4-MU-GAL | 0 | -458 | -464 | -388 | -458 | -477 | -199 | -403 | -504 | -422 | -211 | -452 | -443 | -425 | -196 | -248 | -217 | -71 |
| $10^{-2}$ 4-MU-GAL | 0 | -437 | -434 | -372 | -440 | -437 | -199 | -391 | -434 | -376 | -202 | -430 | -409 | -403 | -171 | -195 | -220 | -34 |
| $10^{-2}$ 4-MU-GAL | 0 | -98 | -135 | -150 | -129 | -125 | -162 | -43 | -147 | -92 | -162 | -199 | -165 | -141 | -238 | -186 | -205 | -25 |
| $10^{-2}$ 4-MU-GAL | 0 | -119 | -165 | -159 | -147 | -110 | -168 | -40 | -141 | -89 | -123 | -162 | -135 | -113 | -208 | -184 | -162 | 76 |
| $10^{-2}$ EHC-GAL | 0 | -705 | -738 | -582 | -732 | -750 | -238 | -647 | -799 | -647 | -296 | -772 | -747 | -726 | -277 | -302 | -296 | -262 |
| $10^{-2}$ EHC-GAL | 0 | -735 | -714 | -601 | -735 | -763 | -219 | -634 | -781 | -647 | -289 | -756 | -753 | -732 | -256 | -323 | -296 | -149 |
| $10^{-2}$ EHC-GAL | 0 | -58 | -110 | -155 | -137 | -131 | -189 | 58 | -134 | -33 | -177 | -219 | -161 | -140 | -329 | -244 | -265 | -52 |
| $10^{-2}$ EHC-GAL | 0 | -22 | -123 | -132 | -132 | -107 | -162 | 70 | -126 | -28 | -162 | -211 | -165 | -126 | -296 | -223 | -251 | -22 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -607 | -635 | -494 | -625 | -650 | -229 | -561 | -668 | -540 | -201 | -647 | -616 | -625 | -244 | -296 | -259 | -207 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -580 | -583 | -476 | -599 | -626 | -202 | -541 | -632 | -525 | -245 | -620 | -614 | -605 | -235 | -257 | -266 | -193 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -73 | -131 | -137 | -137 | -116 | -171 | 28 | -137 | -79 | -164 | -210 | -171 | -131 | -265 | -225 | -238 | -73 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -70 | -137 | -161 | -149 | -113 | -183 | 22 | -146 | -58 | -155 | -192 | -171 | -134 | -268 | -222 | -244 | -52 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -732 | -772 | -604 | -760 | -775 | -204 | -671 | -827 | -665 | -244 | -812 | -775 | -763 | -238 | -311 | -262 | -220 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -720 | -723 | -580 | -726 | -763 | -195 | -644 | -805 | -641 | -256 | -787 | -750 | -747 | -226 | -302 | -287 | -204 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -85 | -192 | -204 | -155 | -152 | -229 | 40 | -165 | -79 | -207 | -259 | -207 | -183 | -378 | -287 | -314 | -67 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -52 | -156 | -195 | -171 | -146 | -211 | 46 | -171 | -46 | -195 | -214 | -186 | -165 | -348 | -266 | -320 | -43 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1721 | -1688 | -1419 | -1648 | -1682 | -754 | -1499 | -1688 | -1435 | -778 | -1612 | -1557 | -1554 | -690 | -794 | -699 | -449 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1539 | -1535 | -1267 | -1490 | -1554 | -647 | -1340 | -1551 | -1313 | -641 | -1502 | -1465 | -1413 | -571 | -653 | -650 | -275 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -299 | -424 | -433 | 3290 | 3543 | 2710 | 1737 | -354 | -241 | -403 | -507 | -378 | -391 | -671 | -549 | -507 | 220 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -323 | -457 | -482 | -433 | -378 | -476 | -85 | -384 | -213 | -412 | -470 | -384 | -332 | -619 | -488 | -497 | 58 |
| $10^{-2}$ control | 0 | -327 | -317 | -256 | -305 | -327 | -104 | -259 | -327 | -272 | -104 | -314 | -302 | -290 | -73 | -98 | -110 | 49 |
| $10^{-2}$ control | 0 | -354 | -348 | -281 | -351 | -342 | -119 | -296 | -345 | -278 | -107 | -342 | -327 | -309 | -95 | -125 | -113 | 52 |
| $10^{-2}$ control | 0 | -49 | -92 | -95 | -80 | -55 | -86 | 27 | -61 | -28 | -71 | -83 | -74 | -49 | -101 | -123 | -104 | 79 |
| $10^{-2}$ control | 0 | -52 | -83 | -83 | -83 | -64 | -89 | 30 | -67 | -12 | -86 | -89 | -61 | -49 | -125 | -98 | -101 | 104 |

## Appendix 5.1: Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| E. coli FRHECO2 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-1}$ 4-MU-GAL | 0 | -427 | -430 | -363 | -445 | -457 | -174 | -378 | -461 | -372 | -226 | -445 | -427 | -415 | -192 | -219 | -210 | -36 |
| $10^{-1}$ 4-MU-GAL | 0 | -434 | -443 | -363 | -427 | -446 | -183 | -382 | -431 | -382 | -192 | -437 | -412 | -400 | -174 | -205 | -177 | -34 |
| $10^{-1}$ 4-MU-GAL | 0 | -104 | -153 | -144 | -141 | -125 | -168 | -46 | -128 | -89 | -171 | -165 | -147 | -128 | -238 | -202 | -220 | -19 |
| $10^{-1}$ 4-MU-GAL | 0 | -112 | -146 | -152 | -143 | -122 | -146 | -42 | -112 | -73 | -122 | -152 | -119 | -106 | -195 | -140 | -161 | 46 |
| $10^{-1}$ EHC-GAL | 0 | -696 | -726 | -558 | -720 | -732 | -195 | -604 | -760 | -610 | -232 | -763 | -708 | -723 | -241 | -323 | -284 | -232 |
| $10^{-1}$ EHC-GAL | 0 | -729 | -741 | -592 | -750 | -787 | -265 | -659 | -805 | -689 | -283 | -784 | -763 | -772 | -283 | -314 | -308 | -247 |
| $10^{-1}$ EHC-GAL | 0 | -98 | -150 | -147 | -156 | -129 | -186 | 33 | -162 | -58 | -177 | -238 | -177 | -141 | -336 | -272 | -263 | -55 |
| $10^{-1}$ EHC-GAL | 0 | -73 | -155 | -180 | -164 | -140 | -210 | 19 | -168 | -64 | -207 | -262 | -201 | -186 | -345 | -268 | -280 | -79 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -580 | -583 | -476 | -592 | -610 | -186 | -522 | -641 | -501 | -229 | -623 | -604 | -562 | -211 | -278 | -269 | -195 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -574 | -583 | -483 | -583 | -614 | -190 | -510 | -620 | -504 | -226 | -626 | -590 | -568 | -214 | -269 | -251 | -150 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -89 | -129 | -150 | -135 | -123 | -156 | 6 | -132 | -52 | -159 | -226 | -153 | -123 | -272 | -223 | -229 | -61 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -92 | -135 | -147 | -144 | -120 | -181 | -7 | -138 | -71 | -190 | -220 | -174 | -156 | -300 | -217 | -229 | -52 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -751 | -763 | -610 | -769 | -781 | -195 | -659 | -830 | -625 | -250 | -815 | -784 | -757 | -256 | -317 | -290 | -238 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -760 | -800 | -650 | -800 | -797 | -263 | -690 | -858 | -687 | -296 | -815 | -812 | -803 | -287 | -330 | -327 | -241 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -62 | -123 | -153 | -144 | -92 | -174 | 70 | -135 | -31 | -171 | -217 | -138 | -113 | -318 | -242 | -254 | -25 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -98 | -156 | -171 | -156 | -137 | -192 | 43 | -177 | -52 | -205 | -235 | -186 | -147 | -351 | -263 | -281 | -43 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1581 | -1617 | -1330 | -1559 | -1623 | -683 | -1373 | -1614 | -1339 | -692 | -1556 | -1501 | -1483 | -604 | -726 | -634 | -317 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1587 | -1596 | -1325 | -1575 | -1596 | -696 | -1389 | -1642 | -1361 | -717 | -1572 | -1523 | -1474 | -635 | -760 | -693 | -321 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -385 | -449 | -476 | -431 | -403 | -488 | -89 | -403 | -244 | -452 | -498 | -421 | -366 | -669 | -522 | -546 | -15 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -440 | -510 | -528 | -516 | -434 | -534 | -128 | -458 | -275 | -479 | -528 | -431 | -403 | -699 | -537 | -559 | -6 |
| $10^{-1}$ control | 0 | -345 | -323 | -268 | -338 | -341 | -119 | -293 | -360 | -283 | -106 | -335 | -332 | -305 | -91 | -122 | -116 | 40 |
| $10^{-1}$ control | 0 | -354 | -376 | -275 | -366 | -351 | -107 | -287 | -357 | -293 | -119 | -333 | -336 | -327 | -101 | -122 | -122 | 76 |
| $10^{-1}$ control | 0 | -104 | -107 | -98 | -101 | -73 | -119 | -9 | -82 | -49 | -82 | -110 | -92 | -58 | -171 | -101 | -92 | 67 |
| $10^{-1}$ control | 0 | -70 | -73 | -76 | -89 | -67 | -104 | -6 | -76 | -21 | -79 | -98 | -64 | -61 | -134 | -79 | -101 | 110 |

## Appendix 5.1: Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| E. coli FRHECO2 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 4-MU-GAL | 0 | -439 | -445 | -372 | -439 | -445 | -186 | -375 | -458 | -372 | -189 | -445 | -436 | -433 | -192 | -207 | -207 | -55 |
| 1 4-MU-GAL | 0 | -412 | -437 | -361 | -422 | -419 | -180 | -376 | -415 | -357 | -184 | -431 | -425 | -382 | -180 | -202 | -190 | -43 |
| 1 4-MU-GAL | 0 | -119 | -122 | -150 | -147 | -128 | -171 | -55 | -128 | -104 | -162 | -180 | -156 | -147 | -244 | -189 | -208 | -6 |
| 1 4-MU-GAL | 0 | -123 | -147 | -177 | -171 | -141 | -174 | -43 | -141 | -71 | -147 | -177 | -156 | -116 | -214 | -165 | -187 | 15 |
| 1 EHC-GAL | 0 | -717 | -769 | -571 | -763 | -778 | -235 | -665 | -839 | -659 | -308 | -803 | -784 | -754 | -314 | -363 | -339 | -284 |
| 1 EHC-GAL | 0 | -739 | -754 | -598 | -748 | -787 | -247 | -693 | -824 | -671 | -308 | -790 | -769 | -775 | -293 | -351 | -323 | -265 |
| 1 EHC-GAL | 0 | -104 | -153 | -177 | -162 | -147 | -202 | 27 | -159 | -55 | -174 | -247 | -211 | -153 | -351 | -269 | -321 | -83 |
| 1 EHC-GAL | 0 | -76 | -122 | -174 | -143 | -113 | -180 | 52 | -134 | -40 | -180 | -229 | -162 | -131 | -320 | -250 | -281 | -40 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -549 | -601 | -470 | -589 | -610 | -195 | -519 | -629 | -510 | -226 | -638 | -595 | -583 | -238 | -275 | -272 | -195 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -586 | -608 | -476 | -598 | -620 | -223 | -550 | -662 | -534 | -275 | -638 | -626 | -611 | -250 | -305 | -287 | -217 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -79 | -110 | -131 | -116 | -94 | -143 | 25 | -119 | -33 | -131 | -201 | -146 | -116 | -284 | -201 | -235 | -46 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -67 | -101 | -131 | -131 | -119 | -165 | 30 | -137 | -67 | -156 | -186 | -147 | -113 | -241 | -195 | -263 | -43 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -787 | -821 | -629 | -793 | -824 | -256 | -717 | -876 | -696 | -320 | -839 | -824 | -812 | -323 | -378 | -372 | -275 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -736 | -760 | -617 | -803 | -806 | -223 | -699 | -846 | -675 | -287 | -815 | -797 | -785 | -275 | -333 | -324 | -257 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -82 | -119 | -167 | -128 | -125 | -183 | 74 | -125 | -15 | -171 | -213 | -128 | -116 | -320 | -222 | -286 | -6 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -89 | -150 | -177 | -153 | -135 | -199 | 55 | -165 | -37 | -196 | -241 | -168 | -147 | -336 | -257 | -293 | -28 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1623 | -1660 | -1337 | -1593 | -1630 | -677 | -1404 | -1648 | -1379 | -699 | -1599 | -1523 | -1483 | -644 | -726 | -693 | -259 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1593 | -1611 | -1321 | -1587 | -1611 | -662 | -1404 | -1648 | -1343 | -705 | -1566 | -1514 | -1480 | -653 | -735 | -708 | -336 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -424 | -436 | -476 | -433 | -396 | -497 | -73 | -403 | -216 | -433 | -500 | -400 | -342 | -671 | -506 | -564 | 31 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -440 | -501 | -538 | -489 | -425 | -528 | -92 | -461 | -248 | -443 | -541 | -416 | -361 | -675 | -516 | -571 | 24 |
| 1control | 0 | -342 | -360 | -277 | -329 | -329 | -143 | -302 | -339 | -271 | -113 | -323 | -320 | -305 | -119 | -119 | -119 | 37 |
| 1control | 0 | -348 | -354 | -287 | -333 | -330 | -113 | -287 | -345 | -269 | -104 | -330 | -315 | -333 | -98 | -116 | -116 | 55 |
| 1control | 0 | -70 | -73 | -91 | -67 | -51 | -82 | -3 | -67 | 4 | -51 | -82 | -45 | -33 | -106 | -79 | -73 | 95 |
| 1control | 0 | -79 | -86 | -86 | -61 | -58 | -89 | -3 | -55 | -15 | -64 | -73 | -43 | -37 | -131 | -89 | -95 | 107 |

## Appendix 5.1: Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| E. coli FRHECO2 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 4-MU-GAL | 0 | -409 | -448 | -378 | -424 | -448 | -192 | -384 | -460 | -381 | -198 | -445 | -412 | -421 | -192 | -222 | -204 | -36 |
| 10 4-MU-GAL | 0 | -415 | -437 | -367 | -406 | -425 | -205 | -388 | -446 | -360 | -196 | -434 | -409 | -400 | -162 | -205 | -202 | -40 |
| 10 4-MU-GAL | 0 | -91 | -116 | -137 | -119 | -103 | -146 | -42 | -128 | -85 | -152 | -164 | -125 | -116 | -219 | -161 | -201 | 4 |
| 10 4-MU-GAL | 0 | -119 | -135 | -156 | -162 | -116 | -150 | -49 | -153 | -92 | -150 | -156 | -141 | -110 | -199 | -159 | -177 | 42 |
| 10 EHC-GAL | 0 | -669 | -742 | -547 | -708 | -745 | -242 | -657 | -788 | -623 | -272 | -745 | -693 | -568 | 555 | 2270 | 6815 | 58925 |
| 10 EHC-GAL | 0 | -729 | -790 | -604 | -751 | -769 | -262 | -702 | -827 | -653 | -302 | -802 | -772 | -784 | -274 | -351 | -335 | -220 |
| 10 EHC-GAL | 0 | -125 | -174 | -183 | -168 | -153 | -229 | 3 | -180 | -88 | -210 | -250 | -220 | -159 | -345 | -278 | -317 | -64 |
| 10 EHC-GAL | 0 | -94 | -168 | -177 | -161 | -107 | -165 | 61 | -146 | -64 | -186 | -229 | -180 | -134 | -348 | -253 | -277 | -33 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -620 | -663 | -507 | -623 | -644 | -242 | -580 | -660 | -538 | -272 | -660 | -632 | -620 | -257 | -318 | -306 | -232 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -592 | -629 | -491 | -604 | -613 | -229 | -558 | -659 | -519 | -256 | -650 | -623 | -619 | -250 | -284 | -278 | -180 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -101 | -116 | -141 | -120 | -123 | -184 | -10 | -116 | -52 | -162 | -174 | -156 | -126 | -266 | -208 | -245 | -31 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -73 | -143 | -149 | -155 | -85 | -177 | 6 | -134 | -79 | -168 | -192 | -140 | -85 | 9 | 1020 | 3208 | 39362 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -715 | -791 | -596 | -760 | -791 | -229 | -690 | -818 | -644 | -278 | -794 | -766 | -754 | -248 | -333 | -287 | -220 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -754 | -812 | -602 | -776 | -791 | -245 | -672 | -843 | -660 | -278 | -821 | -751 | -684 | 167 | 1016 | 3213 | 59495 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -107 | -128 | -162 | -162 | -131 | -214 | 31 | -159 | -55 | -201 | -223 | -162 | -91 | -119 | 476 | 1758 | 48951 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -107 | -171 | -189 | -183 | -140 | -192 | 37 | -192 | -82 | -210 | -247 | -189 | -128 | -351 | -299 | -302 | -33 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1581 | -1666 | -1334 | -1602 | -1611 | -699 | -1410 | -1605 | -1334 | -693 | -1547 | -1480 | -1492 | -607 | -705 | -683 | -287 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1612 | -1682 | -1361 | -1593 | -1639 | -748 | -1438 | -1679 | -1386 | -745 | -1590 | -1514 | -1502 | -473 | -64 | 1150 | 58192 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -427 | -467 | -494 | -473 | -424 | -522 | -143 | -409 | -241 | -458 | -519 | -421 | -366 | -671 | -507 | -583 | 46 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -461 | -509 | -528 | -454 | -442 | -534 | -134 | -454 | -293 | -479 | -543 | -427 | -378 | -683 | -534 | -580 | 61 |
| 10control | 0 | -329 | -342 | -274 | -320 | -314 | -119 | -296 | -342 | -265 | -104 | -320 | -314 | -296 | -94 | -119 | -107 | 52 |
| 10control | 0 | -345 | -351 | -293 | -345 | -336 | -122 | -309 | -354 | -275 | -110 | -342 | -318 | -321 | -98 | -125 | -128 | 45 |
| 10control | 0 | -74 | -83 | -77 | -77 | -52 | -80 | 18 | -61 | -25 | -83 | -80 | -71 | -40 | -113 | -68 | -86 | 97 |
| 10control | 0 | -94 | -97 | -101 | -101 | -58 | -104 | 25 | -67 | -21 | -88 | -94 | -49 | -30 | -146 | -94 | -113 | 101 |

**Appendix 5.1: Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.**

| E. coli FRHECO2 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^2$ 4-MU-GAL | 0 | -443 | -479 | -391 | -449 | -464 | -202 | -421 | -470 | -388 | -205 | -446 | -394 | -315 | 732 | 2658 | 7004 | 42615 |
| $10^2$ 4-MU-GAL | 0 | -415 | -470 | -366 | -437 | -418 | -202 | -403 | -467 | -357 | -171 | -412 | -244 | 6 | 1901 | 4840 | 9605 | 40732 |
| $10^2$ 4-MU-GAL | 0 | -110 | -150 | -150 | -141 | -144 | -156 | -95 | -144 | -98 | -165 | -174 | -132 | -119 | -132 | 131 | 809 | 31902 |
| $10^2$ 4-MU-GAL | 0 | -144 | -153 | -156 | -150 | -132 | -147 | -61 | -153 | -89 | -153 | -174 | -116 | -52 | 201 | 1632 | 4547 | 33636 |
| $10^2$ EHC-GAL | 0 | -677 | -750 | -567 | -720 | -747 | -250 | -674 | -793 | -619 | -265 | -729 | -604 | -320 | 1978 | 6175 | 13460 | 58632 |
| $10^2$ EHC-GAL | 0 | -677 | -744 | -592 | -726 | -763 | -265 | -695 | -805 | -607 | -231 | -613 | -219 | 532 | 5195 | 11378 | 19692 | 57995 |
| $10^2$ EHC-GAL | 0 | -67 | -104 | -134 | -128 | -107 | -177 | -12 | -128 | -30 | -137 | -128 | 186 | 931 | 4001 | 9623 | 15422 | 48258 |
| $10^2$ EHC-GAL | 0 | -55 | -95 | -141 | -116 | -92 | -150 | -4 | -132 | -22 | -150 | -123 | 122 | 567 | 2173 | 6165 | 11905 | 48877 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -583 | -660 | -516 | -617 | -648 | -242 | -586 | -681 | -532 | -269 | -660 | -605 | -501 | 320 | 1153 | 2987 | 47769 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -534 | -568 | -498 | -592 | -607 | -208 | -549 | -656 | -501 | -244 | -607 | -501 | -253 | 1727 | 4648 | 9061 | 47687 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -91 | -122 | -137 | -128 | -106 | -171 | -21 | -116 | -55 | -119 | -164 | -73 | 101 | 547 | 1795 | 3812 | 39814 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -125 | -153 | -190 | -180 | -141 | -177 | -64 | -159 | -98 | -183 | -220 | -122 | 70 | 900 | 3116 | 6168 | 39807 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -754 | -830 | -659 | -808 | -821 | -305 | -766 | -891 | -699 | -317 | -845 | -821 | -769 | -64 | 385 | 2076 | 59523 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -711 | -781 | -595 | -756 | -781 | -250 | -698 | -839 | -653 | -271 | -787 | -763 | -763 | -235 | -305 | -271 | -149 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -113 | -140 | -168 | -119 | -134 | -198 | -18 | -152 | -70 | -192 | -213 | -119 | 25 | 428 | 2402 | 5955 | 49461 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -104 | -140 | -165 | -159 | -95 | -192 | -21 | -140 | -46 | -189 | -174 | -18 | 180 | 601 | 2063 | 4404 | 50669 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1648 | -1751 | -1437 | -1666 | -1709 | -818 | -1529 | -1739 | -1428 | -808 | -1623 | -1571 | -1504 | -167 | 1102 | 4224 | 57378 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1507 | -1587 | -1315 | -1538 | -1574 | -720 | -1431 | -1629 | -1318 | -695 | -1559 | -1483 | -1452 | -537 | -369 | 376 | 57290 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -424 | -485 | -492 | -470 | -434 | -510 | -235 | -470 | -275 | -467 | -516 | -403 | -272 | -232 | 961 | 3101 | 46225 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -452 | -516 | -537 | -488 | -381 | -458 | -189 | -391 | -226 | -458 | -458 | -394 | -314 | -552 | -366 | -195 | 47288 |
| $10^2$ control | 0 | -341 | -348 | -280 | -329 | -326 | -110 | -287 | -329 | -253 | -110 | -326 | -317 | -287 | -82 | -119 | -113 | 77 |
| $10^2$ control | 0 | -342 | -357 | -269 | -330 | -354 | -122 | -312 | -360 | -284 | -116 | -327 | -312 | -309 | -110 | -119 | -125 | -10 |
| $10^2$ control | 0 | -80 | -98 | -92 | -80 | -58 | -92 | 6 | -71 | -22 | -64 | -89 | -61 | -43 | -119 | -83 | -92 | 116 |
| $10^2$ control | 0 | -43 | -67 | -64 | -61 | -46 | -71 | 12 | -40 | -6 | -52 | -67 | -40 | -28 | -95 | -61 | -74 | 146 |

## Appendix 5.1: Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| E. coli FRHECO2 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-3}$ 4-MU-GAL | 0 | -446 | -455 | -379 | -449 | -449 | -205 | -394 | -458 | -373 | -86 | -4 | 2081 | 5997 | 18955 | 27290 | 33163 | 44601 |
| $10^{-3}$ 4-MU-GAL | 0 | -427 | -455 | -363 | -433 | -415 | -213 | -406 | -476 | -357 | -52 | 119 | 2042 | 5137 | 15806 | 22847 | 28994 | 41935 |
| $10^{-3}$ 4-MU-GAL | 0 | -119 | -129 | -138 | -141 | -126 | -150 | -61 | -107 | -19 | -13 | 155 | 1141 | 3476 | 9647 | 17066 | 21904 | 33913 |
| $10^{-3}$ 4-MU-GAL | 0 | -128 | -153 | -174 | -159 | -147 | -168 | -92 | -134 | -83 | -76 | 210 | 2100 | 5616 | 11939 | 18455 | 22447 | 34735 |
| $10^{-3}$ EHC-GAL | 0 | -674 | -754 | -583 | -729 | -741 | -238 | -674 | -803 | -583 | -43 | 37 | 3070 | 8100 | 22902 | 32220 | 39853 | 59316 |
| $10^{-3}$ EHC-GAL | 0 | -677 | -732 | -580 | -735 | -751 | -229 | -684 | -787 | -586 | 28 | 214 | 3482 | 8588 | 24563 | 33725 | 40952 | 59316 |
| $10^{-3}$ EHC-GAL | 0 | -92 | -153 | -180 | -144 | -150 | -192 | -49 | -140 | 6 | 110 | 1047 | 5060 | 10777 | 19014 | 27111 | 31887 | 48573 |
| $10^{-3}$ EHC-GAL | 0 | -103 | -140 | -177 | -168 | -107 | -174 | -21 | -119 | 58 | 348 | 1673 | 6767 | 13512 | 22866 | 30328 | 34485 | 49687 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -570 | -622 | -485 | -592 | -625 | -241 | -570 | -656 | -525 | -198 | -396 | 556 | 2585 | 9782 | 15862 | 22075 | 48664 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -556 | -586 | -461 | -577 | -611 | -217 | -546 | -641 | -482 | -131 | -253 | 980 | 3134 | 11005 | 17012 | 22954 | 48563 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -107 | -144 | -147 | -144 | -141 | -168 | -58 | -119 | -64 | -113 | 21 | 973 | 2942 | 6995 | 12482 | 17347 | 40622 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -104 | -138 | -165 | -156 | -156 | -168 | -40 | -101 | 0 | 58 | 494 | 2353 | 5252 | 10514 | 16157 | 20271 | 40610 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -715 | -757 | -589 | -742 | -766 | -238 | -678 | -815 | -611 | -74 | -238 | 1434 | 4788 | 15211 | 23225 | 31475 | 60151 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -693 | -770 | -596 | -745 | -767 | -220 | -696 | -809 | -605 | -28 | -95 | 2261 | 6354 | 19688 | 27556 | 35143 | 60420 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -88 | -137 | -155 | -146 | -125 | -207 | -33 | -131 | -3 | 12 | 485 | 3000 | 7102 | 13713 | 21166 | 26479 | 49806 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -100 | -149 | -171 | -149 | -128 | -195 | -3 | -122 | 61 | 238 | 1133 | 4603 | 9501 | 17494 | 24673 | 29126 | 50184 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1630 | -1718 | -1358 | -1615 | -1682 | -760 | -1493 | -1694 | -1395 | -723 | -1508 | -858 | 467 | 6788 | 11933 | 17695 | 57680 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1511 | -1609 | -1319 | -1545 | -1590 | -702 | -1423 | -1630 | -1325 | -614 | -1362 | -675 | 659 | 7456 | 12989 | 18061 | 57627 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -442 | -473 | -525 | -479 | -430 | -540 | -271 | -442 | -259 | -427 | -357 | 339 | 1993 | 5927 | 11986 | 17003 | 46778 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -458 | -498 | -495 | -449 | -416 | -498 | -220 | -419 | -202 | -321 | -129 | 1034 | 3006 | 7092 | 11857 | 15308 | 47415 |
| $10^{-3}$ control | 0 | -345 | -370 | -293 | -336 | -336 | -138 | -318 | -351 | -278 | -122 | -360 | -345 | -302 | -95 | -132 | -104 | 55 |
| $10^{-3}$ control | 0 | -342 | -339 | -272 | -324 | -330 | -113 | -300 | -342 | -257 | -116 | -324 | -300 | -306 | -80 | -104 | -89 | 54 |
| $10^{-3}$ control | 0 | -92 | -73 | -80 | -70 | -52 | -86 | -34 | -58 | -25 | -83 | -80 | -61 | -43 | -110 | -80 | -73 | 140 |
| $10^{-3}$ control | 0 | -73 | -89 | -83 | -73 | -61 | -64 | -21 | -58 | -12 | -70 | -86 | -55 | -25 | -101 | -58 | -58 | 98 |

## Appendix 5.1: Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| E. coli FRHECO2 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^4$ 4-MU-GAL | 0 | -446 | -473 | -394 | -452 | -467 | -214 | -409 | -321 | 454 | 5460 | 10306 | 18406 | 23866 | 36221 | 37982 | 38803 | 45731 |
| $10^4$ 4-MU-GAL | 0 | -436 | -448 | -372 | -424 | -439 | -204 | -403 | -409 | 34 | 2805 | 6092 | 13658 | 18816 | 31781 | 34967 | 36899 | 44108 |
| $10^4$ 4-MU-GAL | 0 | -144 | -134 | -159 | -150 | -150 | -156 | -64 | -15 | 552 | 2857 | 7575 | 16169 | 22374 | 25206 | 27526 | 27453 | 35785 |
| $10^4$ 4-MU-GAL | 0 | -122 | -150 | -156 | -128 | -128 | -141 | -77 | -83 | 442 | 3012 | 7679 | 15351 | 21187 | 23961 | 27379 | 28096 | 35925 |
| $10^4$ EHC-GAL | 0 | -711 | -757 | -580 | -714 | -741 | -241 | -622 | -537 | 540 | 6812 | 11305 | 20967 | 26818 | 44492 | 48692 | 50950 | 59084 |
| $10^4$ EHC-GAL | 0 | -696 | -745 | -580 | -705 | -760 | -241 | -638 | -620 | 333 | 5295 | 9513 | 19279 | 26400 | 44349 | 48450 | 51356 | 58620 |
| $10^4$ EHC-GAL | 0 | -92 | -143 | -143 | -122 | -101 | -125 | 37 | 101 | 1370 | 5274 | 11503 | 21150 | 29037 | 33374 | 38376 | 39465 | 49445 |
| $10^4$ EHC-GAL | 0 | -110 | -140 | -162 | -128 | -122 | -152 | 67 | 73 | 1270 | 5161 | 11714 | 21373 | 28829 | 33389 | 38306 | 39380 | 49049 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -589 | -611 | -507 | -596 | -626 | -235 | -556 | -571 | -174 | 2307 | 4394 | 9442 | 14497 | 27599 | 33010 | 37026 | 49784 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -556 | -589 | -473 | -577 | -601 | -229 | -589 | -546 | -34 | 2618 | 4743 | 10520 | 14872 | 27886 | 32723 | 36322 | 48933 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -89 | -119 | -144 | -141 | -132 | -153 | -34 | -49 | 463 | 2063 | 4919 | 10483 | 16316 | 21196 | 26567 | 28862 | 41922 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -110 | -138 | -162 | -135 | -135 | -153 | -6 | -25 | 720 | 2847 | 6564 | 12662 | 18312 | 22392 | 27162 | 29235 | 41067 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -745 | -763 | -601 | -739 | -766 | -226 | -641 | -629 | 183 | 4816 | 7914 | 15745 | 22325 | 39209 | 45337 | 49967 | 60399 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -720 | -750 | -589 | -711 | -741 | -216 | -680 | -586 | 251 | 4841 | 8070 | 16631 | 22323 | 39936 | 45445 | 49864 | 59966 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -119 | -149 | -168 | -161 | -131 | -168 | 25 | 71 | 1139 | 4203 | 9165 | 17269 | 24355 | 29629 | 35904 | 38150 | 50432 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -61 | -122 | -143 | -116 | -91 | -116 | 107 | 202 | 1529 | 5567 | 11577 | 20498 | 27697 | 31949 | 37613 | 39512 | 50911 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1620 | -1657 | -1373 | -1569 | -1617 | -717 | -1471 | -1590 | -1031 | 1221 | 2420 | 6852 | 10823 | 22258 | 27779 | 32360 | 57866 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -1569 | -1633 | -1322 | -1538 | -1578 | -705 | -1438 | -1584 | -989 | 1193 | 2429 | 7517 | 10850 | 23171 | 28039 | 32156 | 57103 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -415 | -470 | -510 | -452 | -427 | -473 | -223 | -382 | -12 | 723 | 2829 | 7871 | 13187 | 17888 | 23180 | 25859 | 47388 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -452 | -473 | -513 | -458 | -403 | -455 | -168 | -333 | 85 | 1068 | 3443 | 8207 | 12553 | 16597 | 21281 | 24358 | 47565 |
| $10^4$ control | 0 | -320 | -317 | -262 | -320 | -332 | -125 | -308 | -332 | -235 | -110 | -305 | -277 | -280 | -67 | -85 | -82 | 34 |
| $10^4$ control | 0 | -317 | -338 | -271 | -317 | -308 | -113 | -302 | -332 | -259 | -85 | -299 | -286 | -277 | -51 | -106 | -88 | -21 |
| $10^4$ control | 0 | -86 | -79 | -95 | -86 | -49 | -92 | -28 | -61 | -15 | -67 | -76 | -58 | -28 | -101 | -61 | -76 | 82 |
| $10^4$ control | 0 | -85 | -91 | -98 | -94 | -76 | -98 | -3 | -76 | -12 | -82 | -104 | -58 | -36 | -113 | -104 | -104 | 70 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

**K. pneumoniae NCTC 10896**

| | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-3}$ 4-MU-GAL | 0 | -40 | 6 | -73 | -101 | -67 | -95 | 6 | -49 | -61 | -88 | -128 | -79 | -95 | -119 | -122 | -95 | 40 |
| $10^{-3}$ 4-MU-GAL | 0 | -92 | -40 | -113 | -141 | -116 | -135 | -68 | -122 | -129 | -171 | -177 | -132 | -126 | -168 | -162 | -165 | -16 |
| $10^{-3}$ 4-MU-GAL | 0 | -24 | -116 | -128 | -116 | -113 | -98 | -21 | -70 | -76 | -128 | -92 | -101 | -46 | -128 | -153 | -116 | 24 |
| $10^{-3}$ 4-MU-GAL | 0 | -12 | -15 | -15 | -18 | -15 | 4 | -3 | -18 | -15 | -9 | -18 | -18 | -15 | -15 | -15 | -9 | 4 |
| $10^{-3}$ EHC-GAL | 0 | -25 | 61 | -144 | -177 | -74 | -162 | 9 | -119 | -119 | -193 | -257 | -165 | -162 | -244 | -251 | -247 | -135 |
| $10^{-3}$ EHC-GAL | 0 | -3 | 91 | -129 | -159 | -83 | -171 | -3 | -116 | -141 | -202 | -257 | -183 | -153 | -245 | -238 | -245 | -119 |
| $10^{-3}$ EHC-GAL | 0 | 70 | -125 | -150 | -131 | -138 | -104 | 42 | -95 | -101 | -211 | -138 | -180 | -116 | -226 | -251 | -202 | -70 |
| $10^{-3}$ EHC-GAL | 0 | 0 | 24 | 0 | -7 | 12 | -7 | 9 | -10 | -3 | -19 | -16 | -7 | -3 | -16 | -3 | -7 | 6 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -36 | 49 | -97 | -134 | -70 | -146 | 0 | -85 | -104 | -168 | -204 | -134 | -155 | -204 | -198 | -189 | -94 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -42 | 34 | -125 | -165 | -58 | -155 | 0 | -104 | -110 | -168 | -213 | -149 | -162 | -201 | -204 | -195 | -100 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 10 | -94 | -143 | -131 | -106 | -82 | 25 | -67 | -76 | -164 | -110 | -149 | -100 | -161 | -198 | -152 | -33 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 7 | 13 | 7 | 7 | -9 | 0 | 0 | 4 | 7 | 7 | -9 | -12 | 7 | -6 | -3 | -6 | 25 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -19 | 91 | -116 | -159 | -77 | -159 | 12 | -95 | -98 | -189 | -254 | -177 | -183 | -235 | -247 | -232 | -107 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -6 | 110 | -113 | -174 | -67 | -155 | 37 | -88 | -94 | -177 | -247 | -180 | -128 | -223 | -238 | -250 | -94 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 92 | -112 | -137 | -116 | -112 | -73 | 83 | -73 | -79 | -183 | -116 | -146 | -94 | -228 | -238 | -174 | -6 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -158 | -152 | -174 | -152 | -149 | -152 | -137 | -152 | -149 | -158 | -149 | -149 | -155 | -155 | -140 | -149 | -143 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -300 | -89 | -406 | -458 | -297 | -425 | -129 | -327 | -370 | -455 | -525 | -379 | -388 | -483 | -501 | -455 | -46 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -278 | -58 | -385 | -458 | -281 | -409 | -110 | -281 | -302 | -421 | -504 | -348 | -351 | -428 | -449 | -418 | -3 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -67 | -372 | -412 | -348 | -314 | -290 | -18 | -235 | -241 | -381 | -284 | -326 | -226 | -421 | -415 | -338 | 74 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 25 | 9 | 12 | 0 | 3 | 9 | 22 | 9 | 9 | 34 | -3 | 12 | 0 | 0 | 3 | 6 | 0 |
| $10^{-3}$ control | 0 | -37 | 15 | -55 | -86 | -37 | -64 | 6 | -49 | -40 | -80 | -101 | -49 | -37 | -77 | -83 | -71 | 70 |
| $10^{-3}$ control | 0 | -34 | 3 | -37 | -73 | -31 | -58 | -3 | -25 | -52 | -58 | -89 | -52 | -46 | -77 | -64 | -67 | 88 |
| $10^{-3}$ control | 0 | -15 | -61 | -82 | -76 | -76 | -42 | 25 | -52 | -33 | -64 | -48 | -64 | -30 | -73 | -85 | -42 | 98 |
| $10^{-3}$ control | 0 | -31 | -80 | -80 | -74 | -68 | -55 | 12 | -34 | -25 | -86 | -52 | -68 | -31 | -95 | -86 | -61 | 88 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| K. pneumoniae NCTC 10896 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-2}$ 4-MU-GAL | 0 | -83 | -19 | -141 | -141 | -110 | -141 | -43 | -116 | -113 | -150 | -141 | -123 | -113 | -165 | -165 | -141 | -13 |
| $10^{-2}$ 4-MU-GAL | 0 | -86 | -43 | -144 | -168 | -104 | -159 | -83 | -129 | -138 | -162 | -181 | -138 | -150 | -162 | -205 | -181 | -43 |
| $10^{-2}$ 4-MU-GAL | 0 | -18 | -116 | -153 | -140 | -134 | -116 | -34 | -73 | -101 | -149 | -107 | -113 | -85 | -159 | -159 | -131 | 34 |
| $10^{-2}$ 4-MU-GAL | 0 | 6 | -6 | 0 | 10 | 3 | 6 | 6 | 0 | -18 | -6 | 19 | 3 | 0 | -3 | 3 | -3 | 13 |
| $10^{-2}$ EHC-GAL | 0 | -9 | 101 | -119 | -140 | -70 | -149 | 52 | -82 | -104 | -171 | -183 | -152 | -125 | -213 | -235 | -195 | -82 |
| $10^{-2}$ EHC-GAL | 0 | -18 | 74 | -122 | -158 | -64 | -152 | -6 | -82 | -125 | -158 | -210 | -167 | -167 | -219 | -222 | -222 | -106 |
| $10^{-2}$ EHC-GAL | 0 | 55 | -135 | -156 | -141 | -126 | -119 | 24 | -89 | -116 | -196 | -141 | -147 | -116 | -238 | -232 | -199 | -68 |
| $10^{-2}$ EHC-GAL | 0 | 3 | -18 | 16 | 22 | 13 | 22 | 13 | 6 | 19 | -12 | 16 | 3 | 16 | -6 | 13 | -3 | 28 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -28 | 40 | -113 | -116 | -52 | -128 | 15 | -89 | -95 | -147 | -168 | -156 | -131 | -195 | -183 | -165 | -92 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 40 | -110 | -128 | -52 | -131 | 15 | -67 | -95 | -150 | -140 | -144 | -122 | -177 | -183 | -168 | -70 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 49 | -113 | -131 | -134 | -110 | -104 | 12 | -70 | -88 | -162 | -113 | -119 | -82 | -174 | -177 | -149 | -61 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 9 | 3 | 9 | 9 | 12 | 9 | 25 | 9 | 12 | 0 | 3 | 19 | 25 | 3 | 12 | 6 | 25 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 6 | 107 | -91 | -137 | -46 | -143 | 55 | -104 | -101 | -174 | -192 | -162 | -149 | -207 | -247 | -217 | -98 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -6 | 95 | -106 | -164 | -67 | -161 | 31 | -85 | -116 | -195 | -216 | -152 | -161 | -216 | -222 | -201 | -79 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 83 | -112 | -143 | -134 | -116 | -76 | 43 | -70 | -76 | -192 | -116 | -152 | -91 | -210 | -225 | -186 | -51 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -165 | -153 | -171 | -159 | -183 | -153 | -168 | -159 | -180 | -180 | -177 | -171 | -168 | -177 | -180 | -180 | -168 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -293 | -77 | -400 | -452 | -309 | -434 | -101 | -312 | -339 | -443 | -510 | -388 | -373 | -464 | -458 | -443 | -52 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -281 | -70 | -397 | -461 | -311 | -409 | -113 | -290 | -327 | -436 | -464 | -378 | -360 | -461 | -473 | -418 | -49 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -70 | -388 | -406 | -363 | -339 | -284 | -9 | -214 | -266 | -382 | -278 | -302 | -208 | -412 | -397 | -314 | 95 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 6 | 6 | -3 | 3 | -15 | 6 | 12 | 12 | -12 | 0 | -15 | 6 | 19 | 3 | 0 | 9 | 9 |
| $10^{-2}$ control | 0 | -27 | 7 | -82 | -70 | -21 | -73 | -6 | -39 | -64 | -58 | -73 | -61 | -36 | -70 | -82 | -67 | 74 |
| $10^{-2}$ control | 0 | -34 | 21 | -52 | -67 | -3 | -77 | 24 | -46 | -40 | -71 | -74 | -46 | -55 | -86 | -80 | -77 | 76 |
| $10^{-2}$ control | 0 | 6 | -64 | -67 | -61 | -64 | -24 | 27 | -43 | -18 | -55 | -40 | -49 | 12 | -67 | -58 | -46 | 92 |
| $10^{-2}$ control | 0 | 9 | -73 | -79 | -70 | -42 | -58 | 22 | -24 | -12 | -70 | -52 | -52 | -21 | -67 | -76 | -49 | 119 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| **K. pneumoniae NCTC 10896** | **0** | **60** | **120** | **180** | **240** | **270** | **300** | **330** | **360** | **390** | **420** | **450** | **480** | **510** | **540** | **570** | **600** | **24h** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-1}$ 4-MU-GAL | 0 | -46 | 0 | -85 | -113 | -76 | -116 | -12 | -73 | -79 | -113 | -113 | -107 | -94 | -137 | -131 | -116 | 25 |
| $10^{-1}$ 4-MU-GAL | 0 | -67 | 6 | -116 | -146 | -98 | -128 | -46 | -98 | -104 | -140 | -143 | -119 | -116 | -137 | -131 | -140 | -18 |
| $10^{-1}$ 4-MU-GAL | 0 | -15 | -119 | -122 | -119 | -98 | -98 | -24 | -70 | -64 | -131 | -98 | -88 | -30 | -64 | 12 | 143 | 34555 |
| $10^{-1}$ 4-MU-GAL | 0 | 9 | 13 | 22 | 0 | -15 | -3 | 0 | 13 | 0 | -12 | 3 | 6 | 9 | 9 | 3 | -6 | 16 |
| $10^{-1}$ EHC-GAL | 0 | -24 | 64 | -152 | -174 | -88 | -183 | -9 | -94 | -131 | -201 | -207 | -161 | -168 | -238 | -241 | -241 | -131 |
| $10^{-1}$ EHC-GAL | 0 | -21 | 64 | -140 | -174 | -82 | -149 | 15 | -91 | -113 | -189 | -213 | -171 | -180 | -232 | -229 | -207 | -125 |
| $10^{-1}$ EHC-GAL | 0 | 71 | -128 | -155 | -149 | -119 | -110 | 49 | -94 | -97 | -186 | -122 | -158 | -100 | -241 | -222 | -195 | -67 |
| $10^{-1}$ EHC-GAL | 0 | 10 | -6 | -9 | 0 | 7 | -9 | 7 | 0 | 10 | -6 | 10 | 3 | 0 | -9 | -3 | -6 | 0 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -34 | 43 | -107 | -116 | -61 | -116 | 6 | -52 | -116 | -171 | -144 | -134 | -125 | -186 | -189 | -192 | -89 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -52 | 76 | -98 | -113 | -49 | -116 | 49 | -98 | -116 | -180 | -189 | -147 | -159 | -220 | -183 | -201 | -95 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -16 | -135 | -177 | -153 | -126 | -116 | 12 | -104 | -110 | -171 | -132 | -156 | -123 | -196 | -193 | -187 | -62 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 12 | 3 | 9 | 22 | 3 | 9 | 19 | 12 | 22 | 0 | -9 | 6 | 6 | 0 | 9 | 3 | -3 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -6 | 86 | -149 | -158 | -70 | -149 | 40 | -103 | -116 | -192 | -186 | -152 | -140 | -229 | -222 | -229 | -116 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -43 | 58 | -138 | -180 | -89 | -162 | 39 | -116 | -129 | -205 | -196 | -174 | -171 | -223 | -238 | -242 | -119 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 68 | -131 | -140 | -116 | -70 | -91 | 68 | -61 | -91 | -164 | -140 | -140 | -76 | -201 | -213 | -177 | -21 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -98 | -122 | -119 | -113 | -122 | -113 | -113 | -125 | -116 | -113 | -129 | -119 | -119 | -113 | -129 | -135 | -101 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -329 | -134 | -455 | -476 | -314 | -449 | -146 | -339 | -369 | -482 | -488 | -378 | -372 | -513 | -497 | -491 | -70 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -305 | -131 | -455 | -510 | -345 | -470 | -119 | -351 | -373 | -492 | -495 | -415 | -397 | -525 | -482 | -485 | -92 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -140 | -424 | -442 | -384 | -369 | -311 | -70 | -265 | -296 | -390 | -317 | -348 | -232 | -439 | -412 | -369 | 110 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 9 | -6 | 0 | 9 | -6 | 3 | 6 | -3 | 9 | 3 | 0 | 16 | 3 | -9 | -3 | 0 | 16 |
| $10^{-1}$ control | 0 | -34 | 0 | -80 | -89 | -22 | -74 | -6 | -52 | -55 | -86 | -95 | -61 | -71 | -92 | -67 | -83 | 27 |
| $10^{-1}$ control | 0 | -49 | 6 | -58 | -92 | -43 | -77 | 18 | -28 | -43 | -64 | -83 | -52 | -49 | -83 | -83 | -70 | 76 |
| $10^{-1}$ control | 0 | -19 | -107 | -92 | -86 | -80 | -62 | -16 | -37 | -46 | -86 | -58 | -40 | -31 | -86 | -101 | -92 | 85 |
| $10^{-1}$ control | 0 | -9 | -88 | -79 | -64 | -82 | -54 | 10 | -45 | -42 | -70 | -48 | -54 | -39 | -94 | -85 | -54 | 110 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| K. pneumoniae NCTC 10896 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 4-MU-GAL | 0 | -76 | -21 | -116 | -128 | -91 | -128 | -15 | -91 | -97 | -125 | -134 | -143 | -116 | -152 | -137 | -146 | 7 |
| 1 4-MU-GAL | 0 | -113 | -55 | -135 | -150 | -104 | -147 | -55 | -101 | -120 | -162 | -159 | -144 | -141 | -168 | -184 | -150 | -43 |
| 1 4-MU-GAL | 0 | -28 | -128 | -134 | -116 | -104 | -92 | -21 | -76 | -79 | -122 | -95 | -107 | -76 | -128 | -104 | -128 | 73 |
| 1 4-MU-GAL | 0 | 15 | 9 | 6 | 15 | 9 | 9 | 12 | 0 | -3 | 12 | 12 | 12 | 25 | -9 | 6 | -9 | 28 |
| 1 EHC-GAL | 0 | -21 | 61 | -122 | -149 | -70 | -140 | 22 | -91 | -110 | -201 | -165 | -155 | -146 | -232 | -229 | -216 | -110 |
| 1 EHC-GAL | 0 | -46 | 61 | -129 | -183 | -83 | -168 | -6 | -126 | -129 | -220 | -190 | -183 | -168 | -248 | -235 | -248 | -138 |
| 1 EHC-GAL | 0 | 58 | -128 | -150 | -122 | -122 | -113 | 30 | -77 | -110 | -205 | -138 | -150 | -110 | -223 | -220 | -190 | -55 |
| 1 EHC-GAL | 0 | -3 | -12 | -3 | 10 | 0 | -3 | 0 | 4 | 7 | 13 | 7 | 4 | 7 | 7 | -18 | -24 | -9 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -43 | 0 | -116 | -153 | -73 | -146 | 3 | -88 | -98 | -153 | -150 | -137 | -137 | -198 | -192 | -198 | -98 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -46 | 55 | -104 | -138 | -52 | -125 | -6 | -92 | -86 | -156 | -162 | -129 | -135 | -205 | -193 | -165 | -104 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 28 | -125 | -122 | -113 | -104 | -85 | -3 | -88 | -116 | -174 | -128 | -134 | -88 | -192 | -177 | -159 | -49 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 7 | 3 | -12 | -9 | 3 | 3 | -6 | 0 | -6 | -9 | -3 | -6 | 3 | 0 | 0 | 0 | 22 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -21 | 98 | -119 | -146 | -36 | -146 | 37 | -94 | -97 | -165 | -165 | -125 | -110 | -213 | -195 | -210 | -88 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -25 | 76 | -138 | -162 | -77 | -153 | 27 | -89 | -110 | -174 | -187 | -153 | -181 | -245 | -229 | -217 | -110 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 55 | -147 | -159 | -129 | -123 | -95 | 55 | -77 | -101 | -199 | -119 | -153 | -83 | -232 | -208 | -190 | -22 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -110 | -143 | -189 | -168 | -192 | -189 | -180 | -192 | -195 | -207 | -198 | -186 | -186 | -192 | -210 | -207 | -168 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -336 | -132 | -452 | -489 | -330 | -443 | -150 | -333 | -373 | -449 | -440 | -388 | -391 | -528 | -513 | -495 | -52 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -299 | -113 | -409 | -473 | -286 | -421 | -119 | -299 | -335 | -448 | -436 | -366 | -351 | -488 | -454 | -442 | -27 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -94 | -415 | -402 | -351 | -320 | -268 | -30 | -238 | -250 | -406 | -311 | -314 | -225 | -393 | -375 | -314 | 138 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 18 | 15 | 15 | 9 | 15 | 18 | 25 | -27 | 6 | 15 | 21 | 37 | 15 | 9 | 15 | 15 | 34 |
| 1control | 0 | -39 | 13 | -67 | -67 | -24 | -48 | 28 | -24 | -58 | -64 | -64 | -55 | -27 | -85 | -67 | -55 | 58 |
| 1control | 0 | -52 | 15 | -58 | -92 | -28 | -76 | 15 | -34 | -46 | -95 | -64 | -61 | -34 | -104 | -83 | -92 | 67 |
| 1control | 0 | -18 | -94 | -97 | -79 | -64 | -64 | 0 | -48 | -58 | -82 | -58 | -61 | -33 | -88 | -91 | -76 | 74 |
| 1control | 0 | 28 | -100 | -64 | -61 | -64 | -61 | 25 | -24 | -42 | -70 | -48 | -67 | -27 | -85 | -88 | -64 | 110 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| K. pneumoniae NCTC 10896 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 4-MU-GAL | 0 | -52 | -22 | -113 | -120 | -80 | -120 | -25 | -95 | -107 | -141 | -110 | -113 | -104 | -147 | -120 | -135 | 15 |
| 10 4-MU-GAL | 0 | -119 | -64 | -162 | -180 | -125 | -159 | -76 | -137 | -144 | -174 | -183 | -156 | -150 | -183 | -186 | -165 | -49 |
| 10 4-MU-GAL | 0 | -18 | -116 | -159 | -137 | -110 | -85 | -18 | -70 | -98 | -125 | -79 | -88 | -6 | 24 | 177 | 430 | 36413 |
| 10 4-MU-GAL | 0 | 0 | -6 | -21 | -9 | -6 | -12 | 10 | -6 | -15 | -15 | -24 | -24 | 13 | -15 | -3 | 0 | 19 |
| 10 EHC-GAL | 0 | -33 | 55 | -119 | -152 | -67 | -146 | 46 | -97 | -106 | -167 | -158 | -109 | -88 | -54 | 168 | 522 | 45817 |
| 10 EHC-GAL | 0 | -25 | 58 | -138 | -168 | -86 | -156 | 33 | -107 | -138 | -180 | -186 | -153 | -138 | -162 | -31 | 198 | 45777 |
| 10 EHC-GAL | 0 | 64 | -113 | -150 | -131 | -110 | -92 | 40 | -86 | -98 | -171 | -98 | -107 | 3 | -52 | 195 | 415 | 46747 |
| 10 EHC-GAL | 0 | 27 | 24 | 12 | 21 | 12 | 18 | 18 | 24 | 18 | 21 | 15 | 21 | 31 | 18 | 21 | 15 | 15 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -39 | 43 | -88 | -110 | -46 | -122 | 34 | -76 | -88 | -119 | -140 | -79 | -46 | 31 | 260 | 666 | 37830 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -49 | 12 | -125 | -158 | -85 | -143 | 3 | -100 | -116 | -155 | -165 | -128 | -116 | -152 | -116 | -15 | 37451 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 9 | -122 | -146 | -137 | -131 | -113 | -18 | -91 | -100 | -164 | -110 | -100 | 28 | 119 | 513 | 1160 | 38309 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 6 | -15 | 13 | -12 | 13 | -9 | 13 | 10 | -12 | 0 | -21 | -24 | -6 | -18 | -3 | -18 | 6 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -67 | 70 | -149 | -177 | -85 | -174 | 21 | -116 | -125 | -204 | -198 | -180 | -174 | -262 | -232 | -241 | -67 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -52 | 64 | -134 | -198 | -92 | -171 | 18 | -134 | -153 | -205 | -205 | -168 | -153 | -183 | -131 | 6 | 46522 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 80 | -97 | -152 | -119 | -79 | -67 | 77 | -58 | -94 | -158 | -122 | -131 | -30 | -103 | 71 | 260 | 47493 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -49 | -71 | -101 | -74 | -113 | -107 | -95 | -116 | -128 | -122 | -119 | -113 | -119 | -128 | -113 | -119 | -104 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -342 | -165 | -482 | -491 | -366 | -467 | -161 | -354 | -381 | -491 | -448 | -421 | -406 | -470 | -345 | -174 | 44224 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -293 | -110 | -418 | -482 | -320 | -418 | -110 | -332 | -348 | -436 | -430 | -366 | -366 | -476 | -442 | -375 | 43876 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -122 | -369 | -433 | -376 | -342 | -284 | -64 | -260 | -269 | -406 | -314 | -321 | -235 | -424 | -394 | -357 | 201 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 6 | 10 | 6 | 0 | 0 | 6 | -3 | 0 | -3 | 3 | -21 | 6 | 0 | 3 | 3 | 10 | 13 |
| 10control | 0 | -67 | -12 | -92 | -89 | -46 | -73 | 0 | -49 | -64 | -104 | -92 | -70 | -61 | -98 | -92 | -89 | 140 |
| 10control | 0 | -46 | 6 | -95 | -70 | -37 | -70 | 24 | -40 | -49 | -83 | -55 | -70 | -55 | -79 | -83 | -79 | 159 |
| 10control | 0 | -21 | -82 | -82 | -82 | -76 | -55 | 0 | -52 | -55 | -79 | -73 | -64 | -36 | -100 | -79 | -61 | 202 |
| 10control | 0 | -31 | -74 | -89 | -86 | -58 | -34 | 6 | -40 | -55 | -74 | -58 | -40 | -28 | -80 | -83 | -49 | 85 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| **K. pneumoniae NCTC 10896** | **0** | **60** | **120** | **180** | **240** | **270** | **300** | **330** | **360** | **390** | **420** | **450** | **480** | **510** | **540** | **570** | **600** | **24h** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^2$ 4-MU-GAL | 0 | -79 | -33 | -119 | -140 | -91 | -119 | -36 | -91 | -100 | -103 | -61 | -3 | 107 | 388 | 898 | 1710 | 36142 |
| $10^2$ 4-MU-GAL | 0 | -110 | -68 | -156 | -165 | -129 | -168 | -71 | -126 | -113 | -147 | -86 | 6 | 100 | 439 | 1013 | 1739 | 33184 |
| $10^2$ 4-MU-GAL | 0 | -48 | -137 | -170 | -140 | -137 | -143 | -48 | -91 | -137 | -143 | -103 | -70 | 0 | 86 | 348 | 773 | 36832 |
| $10^2$ 4-MU-GAL | 0 | 37 | 15 | 21 | 24 | 15 | 15 | 12 | 27 | 18 | 37 | 18 | 18 | 34 | 24 | 18 | 27 | 31 |
| $10^2$ EHC-GAL | 0 | -33 | 61 | -134 | -159 | -73 | -159 | 34 | -76 | -94 | -101 | -9 | 284 | 565 | 1850 | 3879 | 6550 | 45673 |
| $10^2$ EHC-GAL | 0 | -33 | 74 | -140 | -155 | -79 | -189 | 25 | -94 | -94 | -116 | 0 | 342 | 669 | 2259 | 4423 | 7377 | 45710 |
| $10^2$ EHC-GAL | 0 | 49 | -165 | -177 | -147 | -156 | -128 | -6 | -98 | -101 | -147 | 3 | 271 | 662 | 1733 | 3561 | 6314 | 46054 |
| $10^2$ EHC-GAL | 0 | 10 | -18 | 0 | 16 | -15 | 0 | 13 | 10 | 6 | 3 | 10 | -3 | 6 | 10 | -6 | 13 | 19 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -40 | 46 | -104 | -144 | -55 | -113 | 18 | -73 | -89 | -131 | -95 | 9 | 58 | 406 | 1077 | 2069 | 37948 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -64 | -6 | -138 | -168 | -92 | -168 | -19 | -132 | -153 | -159 | -141 | -67 | 12 | 268 | 866 | 1721 | 37780 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 15 | -101 | -149 | -140 | -122 | -101 | 3 | -73 | -98 | -165 | -91 | -73 | 70 | 287 | 882 | 1880 | 38678 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -12 | -22 | -34 | -22 | -25 | -15 | -22 | -12 | -12 | -37 | -25 | -19 | -28 | -12 | -25 | -25 | 3 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -49 | 79 | -147 | -171 | -92 | -153 | 33 | -83 | -113 | -171 | -110 | -18 | 94 | 464 | 1291 | 2475 | 47046 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -30 | 89 | -128 | -149 | -64 | -164 | 52 | -131 | -106 | -140 | -116 | -15 | 98 | 486 | 1224 | 2259 | 46681 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 61 | -122 | -159 | -125 | -98 | -107 | 36 | -73 | -80 | -150 | -31 | 104 | 467 | 1163 | 2850 | 5405 | 47693 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -85 | -147 | -159 | -177 | -177 | -195 | -180 | -174 | -195 | -192 | -195 | -192 | -189 | -186 | -192 | -198 | -165 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -303 | -120 | -434 | -455 | -345 | -455 | -129 | -336 | -355 | -471 | -403 | -290 | -229 | 45 | 695 | 1797 | 44015 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -330 | -116 | -446 | -480 | -327 | -446 | -119 | -339 | -364 | -458 | -406 | -358 | -324 | -312 | -104 | 201 | 43338 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -141 | -415 | -455 | -385 | -336 | -309 | -77 | -257 | -287 | -424 | -287 | -309 | -186 | -305 | -119 | 226 | 43869 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 6 | 3 | 3 | 0 | -6 | 6 | -9 | 9 | -3 | 9 | -6 | -6 | 3 | 6 | 0 | 3 | 19 |
| $10^2$ control | 0 | -61 | 6 | -79 | -89 | -34 | -83 | 9 | -46 | -55 | -76 | -83 | -76 | -58 | -79 | -83 | -83 | 171 |
| $10^2$ control | 0 | -46 | 0 | -83 | -86 | -37 | -92 | 15 | -37 | -21 | -92 | -83 | -49 | -67 | -98 | -73 | -76 | 159 |
| $10^2$ control | 0 | -24 | -100 | -103 | -79 | -73 | -55 | 13 | -42 | -48 | -103 | -61 | -70 | -42 | -79 | -73 | -67 | 223 |
| $10^2$ control | 0 | -3 | -61 | -67 | -67 | -45 | -33 | 9 | -21 | -33 | -64 | -33 | -42 | -6 | -64 | -64 | -52 | 180 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| **K. pneumoniae NCTC 10896** | **0** | **60** | **120** | **180** | **240** | **270** | **300** | **330** | **360** | **390** | **420** | **450** | **480** | **510** | **540** | **570** | **600** | **24h** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^3$ 4-MU-GAL | 0 | -86 | -46 | -147 | -147 | -107 | -134 | -9 | -31 | 49 | 259 | 723 | 1822 | 3217 | 6754 | 11118 | 15666 | 36798 |
| $10^3$ 4-MU-GAL | 0 | -116 | -76 | -171 | -189 | -146 | -171 | -61 | -98 | -40 | 131 | 409 | 1175 | 1923 | 4151 | 7004 | 10078 | 34732 |
| $10^3$ 4-MU-GAL | 0 | -30 | -131 | -140 | -113 | -116 | -94 | -6 | -6 | 83 | 324 | 779 | 1734 | 3370 | 6260 | 10340 | 14693 | 37772 |
| $10^3$ 4-MU-GAL | 0 | 12 | -3 | 0 | 9 | 9 | 3 | 16 | 0 | 12 | -3 | 19 | 9 | 6 | -9 | -6 | 12 | 16 |
| $10^3$ EHC-GAL | 0 | -22 | 79 | -135 | -165 | -80 | -132 | 64 | -19 | 85 | 534 | 1202 | 3094 | 5155 | 11701 | 19261 | 28881 | 46378 |
| $10^3$ EHC-GAL | 0 | -55 | 67 | -137 | -165 | -101 | -153 | 64 | -21 | 101 | 534 | 1343 | 3342 | 5695 | 12547 | 20516 | 29766 | 46372 |
| $10^3$ EHC-GAL | 0 | 37 | -125 | -158 | -165 | -128 | -125 | 73 | -6 | 168 | 629 | 1609 | 3812 | 6699 | 13102 | 21450 | 31222 | 47172 |
| $10^3$ EHC-GAL | 0 | 46 | 33 | 39 | 36 | 43 | 43 | 39 | 36 | 43 | 43 | 33 | 36 | 39 | 33 | 24 | 39 | 58 |
| $10^3$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -55 | 43 | -103 | -140 | -76 | -143 | 10 | -64 | -55 | 68 | 284 | 895 | 1710 | 4945 | 9544 | 15288 | 39100 |
| $10^3$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -33 | 55 | -116 | -140 | -58 | -98 | 64 | -36 | 12 | 134 | 452 | 1288 | 2295 | 6077 | 11347 | 18605 | 38919 |
| $10^3$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 13 | -125 | -143 | -128 | -131 | -91 | 9 | -49 | 6 | 113 | 498 | 1362 | 2680 | 6031 | 11415 | 20015 | 40058 |
| $10^3$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -3 | -3 | -12 | 22 | 10 | -3 | -12 | -21 | -3 | 7 | -6 | 22 | 0 | -21 | -27 | -9 | 10 |
| $10^3$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -43 | 73 | -150 | -162 | -79 | -171 | 58 | -24 | 15 | 284 | 702 | 1929 | 3375 | 8384 | 14927 | 24395 | 47352 |
| $10^3$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -67 | 101 | -149 | -186 | -94 | -183 | 58 | -39 | 31 | 366 | 971 | 2503 | 4288 | 9901 | 16826 | 26211 | 47282 |
| $10^3$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 31 | -122 | -140 | -146 | -125 | -94 | 83 | -3 | 95 | 345 | 1059 | 2494 | 4578 | 9718 | 16624 | 26394 | 48322 |
| $10^3$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -18 | -61 | -76 | -64 | -76 | -64 | -73 | -76 | -67 | -85 | -61 | -82 | -76 | -73 | -73 | -73 | -55 |
| $10^3$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -317 | -104 | -427 | -443 | -317 | -461 | -98 | -311 | -305 | -281 | -61 | 433 | 1025 | 3116 | 6461 | 10719 | 44455 |
| $10^3$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -299 | -122 | -452 | -488 | -317 | -442 | -94 | -278 | -305 | -302 | -201 | 116 | 440 | 1648 | 3678 | 6617 | 43989 |
| $10^3$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -128 | -403 | -434 | -400 | -379 | -314 | -61 | -247 | -244 | -314 | -67 | 259 | 1016 | 2188 | 4914 | 8872 | 44352 |
| $10^3$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 3 | -25 | -7 | -19 | -3 | -10 | -13 | 0 | -22 | -16 | -7 | -19 | -13 | -16 | -19 | -22 | 9 |
| $10^3$ control | 0 | -37 | 15 | -80 | -89 | -37 | -73 | 18 | -37 | -46 | -70 | -67 | -64 | -64 | -49 | -58 | -43 | 201 |
| $10^3$ control | 0 | -58 | 9 | -95 | -85 | -52 | -88 | 12 | -67 | -61 | -73 | -85 | -70 | -61 | -82 | -76 | -58 | 159 |
| $10^3$ control | 0 | -36 | -64 | -76 | -73 | -55 | -46 | 22 | -33 | -33 | -67 | -61 | -46 | -18 | -70 | -55 | -33 | 214 |
| $10^3$ control | 0 | -18 | -88 | -85 | -64 | -61 | -51 | 25 | -36 | -30 | -73 | -27 | -39 | -24 | -88 | -67 | -64 | 138 |

## Appendix 5.1: Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| K. pneumoniae NCTC 10896 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^4$ 4-MU-GAL | 0 | -107 | -28 | -138 | -101 | -37 | 9 | 357 | 738 | 1785 | 4150 | 7581 | 13196 | 17799 | 22774 | 25490 | 26753 | 37286 |
| $10^4$ 4-MU-GAL | 0 | -116 | -71 | -171 | -165 | -83 | -52 | 259 | 546 | 1361 | 3204 | 5722 | 10010 | 13709 | 18672 | 23100 | 26018 | 37191 |
| $10^4$ 4-MU-GAL | 0 | -46 | -116 | -128 | -92 | -61 | 15 | 293 | 522 | 1221 | 3009 | 5805 | 10310 | 14665 | 19313 | 23241 | 25548 | 37527 |
| $10^4$ 4-MU-GAL | 0 | -3 | -10 | -19 | -13 | -10 | -19 | -19 | -25 | -16 | -31 | -22 | -13 | -19 | -25 | -10 | -25 | -7 |
| $10^4$ EHC-GAL | 0 | -28 | 97 | -125 | -116 | 9 | 21 | 543 | 1047 | 2591 | 6232 | 11020 | 19795 | 28798 | 36499 | 39651 | 40613 | 46244 |
| $10^4$ EHC-GAL | 0 | -31 | 76 | -116 | -123 | 9 | 12 | 497 | 967 | 2432 | 6146 | 11066 | 19996 | 28746 | 36361 | 39413 | 40588 | 45972 |
| $10^4$ EHC-GAL | 0 | 55 | -143 | -180 | -106 | -52 | 61 | 565 | 1117 | 2842 | 7249 | 13338 | 23257 | 33325 | 37766 | 39914 | 40864 | 47059 |
| $10^4$ EHC-GAL | 0 | -18 | -18 | -31 | -15 | -43 | -31 | -9 | -6 | -22 | -6 | -22 | -28 | -25 | -15 | -25 | -18 | -15 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -46 | 30 | -135 | -138 | -58 | -92 | 195 | 320 | 882 | 2627 | 5475 | 11603 | 19432 | 27132 | 30925 | 32833 | 40011 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -61 | 37 | -119 | -128 | -36 | -82 | 223 | 363 | 1065 | 3037 | 6297 | 13066 | 20189 | 27117 | 30734 | 32538 | 39203 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 30 | -101 | -135 | -104 | -55 | -3 | 232 | 387 | 1098 | 3149 | 6623 | 13560 | 22188 | 28222 | 31600 | 33501 | 40967 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 6 | 16 | -6 | 9 | -3 | 13 | 6 | 6 | 0 | 0 | -3 | -9 | 6 | 0 | 0 | 0 | 19 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -37 | 85 | -131 | -110 | -3 | -22 | 421 | 693 | 1773 | 4563 | 8750 | 17381 | 27221 | 35928 | 39725 | 41153 | 47684 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -10 | 106 | -116 | -113 | 12 | 3 | 460 | 793 | 1971 | 4880 | 9238 | 17454 | 27062 | 35452 | 39340 | 40799 | 47034 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 46 | -116 | -183 | -113 | -67 | 12 | 397 | 690 | 1804 | 4832 | 9315 | 17653 | 28085 | 35602 | 39218 | 40906 | 48228 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -70 | -119 | -137 | -122 | -137 | -122 | -125 | -125 | -128 | -137 | -122 | -116 | -122 | -137 | -137 | -156 | -113 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -299 | -92 | -415 | -440 | -284 | -397 | 55 | -37 | 317 | 1330 | 3165 | 7413 | 11820 | 19645 | 26070 | 29784 | 44211 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -318 | -113 | -434 | -473 | -296 | -409 | 21 | -58 | 290 | 1099 | 2570 | 6086 | 9867 | 17756 | 25035 | 29378 | 45017 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -110 | -373 | -431 | -376 | -318 | -254 | 64 | -19 | 308 | 1159 | 3052 | 6668 | 11213 | 17808 | 24867 | 28344 | 44797 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 30 | 33 | 27 | 24 | 21 | 33 | 33 | 36 | 27 | 24 | 30 | 24 | 33 | 30 | 36 | 36 | 49 |
| $10^4$ control | 0 | -49 | 18 | -61 | -77 | -25 | -77 | 27 | -40 | -37 | -64 | -64 | -40 | -43 | -64 | -52 | -74 | 183 |
| $10^4$ control | 0 | -28 | 27 | -61 | -55 | -13 | -61 | 51 | -25 | -19 | -58 | -55 | -25 | -25 | -64 | -55 | -49 | 119 |
| $10^4$ control | 0 | -21 | -79 | -82 | -67 | -61 | -51 | 19 | -36 | -51 | -79 | -61 | -51 | -12 | -70 | -70 | -58 | 177 |
| $10^4$ control | 0 | 13 | -55 | -64 | -70 | -45 | -42 | 19 | -21 | -45 | -61 | -21 | -52 | -18 | -70 | -64 | -42 | 125 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

**C. freundii NCTC 9750**

| | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-3}$ 4-MU-GAL | 0 | -83 | -135 | -119 | -122 | -138 | -168 | -107 | -144 | -135 | -95 | -116 | -150 | -153 | -196 | -196 | -147 | 116 |
| $10^{-3}$ 4-MU-GAL | 0 | -48 | -131 | -100 | -100 | -137 | -116 | -73 | -134 | -119 | -79 | -100 | -106 | -128 | -155 | -155 | -128 | 122 |
| $10^{-3}$ 4-MU-GAL | 0 | -123 | -123 | -113 | -129 | -123 | -126 | -95 | -120 | -138 | -113 | -177 | -144 | -156 | -187 | -150 | -110 | 137 |
| $10^{-3}$ 4-MU-GAL | 0 | -146 | -134 | -128 | -122 | -113 | -119 | -113 | -116 | -116 | -113 | -158 | -106 | -146 | -161 | -116 | -88 | 174 |
| $10^{-3}$ EHC-GAL | 0 | 12 | -129 | -135 | -119 | -187 | -183 | -74 | -205 | -159 | -98 | -177 | -223 | -223 | -290 | -281 | -232 | 106 |
| $10^{-3}$ EHC-GAL | 0 | 3 | 9 | 6 | -4 | -13 | 6 | -4 | -4 | 0 | -10 | -4 | 0 | -4 | -16 | -22 | -10 | 9 |
| $10^{-3}$ EHC-GAL | 0 | -134 | -131 | -180 | -128 | -152 | -143 | -112 | -122 | -149 | -140 | -268 | -140 | -198 | -250 | -192 | -164 | 132 |
| $10^{-3}$ EHC-GAL | 0 | -138 | -135 | -168 | -162 | -183 | -193 | -110 | -144 | -174 | -186 | -254 | -183 | -211 | -260 | -202 | -180 | 116 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 12 | -116 | -101 | -107 | -144 | -156 | -76 | -137 | -134 | -49 | -134 | -159 | -165 | -208 | -217 | -180 | 107 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -18 | -131 | -122 | -110 | -155 | -161 | -82 | -174 | -158 | -82 | -152 | -183 | -195 | -241 | -232 | -210 | 70 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -101 | -92 | -156 | -138 | -113 | -122 | -70 | -107 | -125 | -122 | -208 | -125 | -165 | -214 | -153 | -122 | 113 |
| $10^{-3}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -107 | -104 | -141 | -95 | -116 | -116 | -83 | -98 | -116 | -107 | -180 | -107 | -144 | -177 | -138 | -110 | 131 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 27 | -134 | -150 | -144 | -180 | -183 | -80 | -180 | -171 | -98 | -159 | -223 | -235 | -266 | -281 | -257 | 122 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 18 | -165 | -134 | -150 | -205 | -192 | -101 | -205 | -208 | -141 | -196 | -263 | -272 | -327 | -330 | -284 | 46 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -134 | -125 | -167 | -161 | -140 | -134 | -91 | -103 | -161 | -146 | -256 | -152 | -216 | -232 | -183 | -161 | 156 |
| $10^{-3}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -152 | -125 | -155 | -128 | -128 | -134 | -88 | -112 | -122 | -125 | -228 | -128 | -189 | -225 | -158 | -137 | 165 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -140 | -397 | -357 | -323 | -412 | -400 | -198 | -369 | -354 | -180 | -268 | -351 | -369 | -442 | -430 | -387 | 427 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -220 | -427 | -354 | -357 | -452 | -409 | -269 | -394 | -354 | -204 | -308 | -385 | -406 | -473 | -482 | -394 | 363 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -397 | -379 | -421 | -360 | -369 | -357 | -278 | -266 | -314 | -290 | -488 | -25 | -238 | -388 | -314 | -256 | 482 |
| $10^{-3}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -424 | -375 | -433 | -360 | -369 | -332 | -229 | -259 | -293 | -290 | -470 | -268 | -372 | -418 | -317 | -262 | 470 |
| $10^{-3}$ control | 0 | -34 | -61 | -61 | -67 | -95 | -80 | -61 | -95 | -76 | -25 | -64 | -76 | -80 | -104 | -107 | -86 | 183 |
| $10^{-3}$ control | 0 | -12 | -86 | -61 | -70 | -76 | -67 | -46 | -73 | -67 | -22 | -46 | -92 | -104 | -95 | -101 | -58 | 189 |
| $10^{-3}$ control | 0 | -92 | -77 | -71 | -55 | -43 | -49 | -40 | -55 | -80 | -62 | -98 | -40 | -65 | -80 | -77 | -40 | 216 |
| $10^{-3}$ control | 0 | -68 | -80 | -71 | -61 | -55 | -46 | -40 | -40 | -58 | -52 | -95 | -43 | -77 | -95 | -52 | -16 | 225 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| C. freundii NCTC 9750 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-2}$ 4-MU-GAL | 0 | -64 | -132 | -116 | -110 | -153 | -144 | -98 | -132 | -132 | -71 | -116 | -144 | -156 | -153 | -150 | -147 | 149 |
| $10^{-2}$ 4-MU-GAL | 0 | -64 | -146 | -106 | -91 | -146 | -146 | -100 | -119 | -116 | -67 | -116 | -128 | -131 | -152 | -177 | -131 | 123 |
| $10^{-2}$ 4-MU-GAL | 0 | -109 | -116 | -143 | -137 | -116 | -125 | -109 | -119 | -137 | -112 | -173 | -116 | -143 | -167 | -134 | -103 | 141 |
| $10^{-2}$ 4-MU-GAL | 0 | -116 | -119 | -128 | -128 | -137 | -107 | -82 | -104 | -116 | -134 | -174 | -92 | -125 | -140 | -113 | -107 | 156 |
| $10^{-2}$ EHC-GAL | 0 | 25 | -128 | -101 | -140 | -171 | -177 | -58 | -168 | -159 | -52 | -128 | -192 | -183 | -250 | -253 | -198 | 125 |
| $10^{-2}$ EHC-GAL | 0 | 13 | 7 | 0 | -30 | -9 | 7 | -3 | -9 | 4 | 0 | -18 | 16 | -3 | -15 | -9 | 0 | 0 |
| $10^{-2}$ EHC-GAL | 0 | -155 | -131 | -159 | -128 | -110 | -125 | -76 | -76 | -119 | -122 | -226 | -116 | -162 | -213 | -159 | -119 | 186 |
| $10^{-2}$ EHC-GAL | 0 | -119 | -137 | -165 | -149 | -152 | -143 | -70 | -116 | -143 | -146 | -256 | -146 | -183 | -232 | -168 | -155 | 122 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -21 | -137 | -119 | -104 | -171 | -159 | -73 | -140 | -134 | -82 | -156 | -171 | -159 | -205 | -217 | -177 | 110 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -3 | -135 | -101 | -107 | -138 | -153 | -67 | -144 | -135 | -67 | -113 | -177 | -162 | -208 | -214 | -174 | 107 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -125 | -101 | -159 | -131 | -116 | -128 | -98 | -116 | -144 | -116 | -205 | -125 | -168 | -214 | -159 | -138 | 113 |
| $10^{-2}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -107 | -79 | -122 | -110 | -107 | -98 | -55 | -76 | -86 | -110 | -205 | -104 | -150 | -177 | -147 | -110 | 101 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 13 | -131 | -122 | -113 | -183 | -174 | -73 | -158 | -161 | -58 | -140 | -201 | -198 | -256 | -262 | -207 | 153 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 37 | -128 | -109 | -109 | -180 | -170 | -85 | -167 | -158 | -67 | -143 | -210 | -207 | -268 | -274 | -219 | 156 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -149 | -137 | -164 | -137 | -161 | -155 | -100 | -109 | -167 | -167 | -274 | -149 | -198 | -247 | -192 | -155 | 144 |
| $10^{-2}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -119 | -119 | -143 | -162 | -140 | -128 | -70 | -104 | -128 | -125 | -259 | -131 | -168 | -226 | -162 | -140 | 177 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -165 | -397 | -339 | -327 | -400 | -391 | -205 | -345 | -311 | -192 | -263 | -342 | -366 | -436 | -440 | -351 | 446 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -259 | -479 | -415 | -360 | -463 | -421 | -271 | -409 | -375 | -228 | -320 | -378 | -409 | -463 | -448 | -363 | 385 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -403 | -338 | -400 | -342 | -348 | -326 | -222 | -250 | -293 | -259 | -491 | -113 | -268 | -357 | -244 | -180 | 577 |
| $10^{-2}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -364 | -321 | -373 | -318 | -315 | -260 | -193 | -211 | -263 | -235 | -428 | -220 | -321 | -391 | -272 | -248 | 457 |
| $10^{-2}$ control | 0 | -34 | -83 | -49 | -74 | -86 | -86 | -28 | -68 | -77 | -16 | -77 | -95 | -55 | -80 | -92 | -58 | 177 |
| $10^{-2}$ control | 0 | -46 | -83 | -79 | -67 | -119 | -92 | -49 | -83 | -64 | -40 | -64 | -73 | -95 | -95 | -104 | -86 | 201 |
| $10^{-2}$ control | 0 | -82 | -61 | -67 | -64 | -42 | -67 | -33 | -30 | -70 | -58 | -94 | -33 | -58 | -76 | -52 | -27 | 193 |
| $10^{-2}$ control | 0 | -77 | -61 | -71 | -58 | -71 | -74 | -43 | -40 | -58 | -52 | -95 | -28 | -43 | -28 | -25 | 3 | 259 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| C. freundii NCTC 9750 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-1}$ 4-MU-GAL | 0 | -42 | -94 | -116 | -109 | -125 | -137 | -76 | -94 | -109 | -64 | -85 | -125 | -109 | -155 | -164 | -131 | 156 |
| $10^{-1}$ 4-MU-GAL | 0 | -77 | -171 | -156 | -138 | -162 | -159 | -113 | -141 | -138 | -104 | -135 | -147 | -147 | -171 | -180 | -156 | 97 |
| $10^{-1}$ 4-MU-GAL | 0 | -116 | -112 | -137 | -137 | -109 | -112 | -103 | -119 | -116 | -106 | -180 | -109 | -131 | -149 | -134 | -103 | 156 |
| $10^{-1}$ 4-MU-GAL | 0 | -125 | -128 | -143 | -125 | -128 | -119 | -97 | -107 | -113 | -116 | -165 | -100 | -122 | -149 | -119 | -107 | 128 |
| $10^{-1}$ EHC-GAL | 0 | 0 | -143 | -131 | -113 | -162 | -180 | -94 | -155 | -159 | -73 | -146 | -213 | -204 | -253 | -250 | -213 | 128 |
| $10^{-1}$ EHC-GAL | 0 | 10 | -6 | 3 | 6 | 0 | 0 | 10 | 3 | 10 | -6 | 16 | 3 | 28 | -12 | -15 | 3 | 19 |
| $10^{-1}$ EHC-GAL | 0 | -134 | -128 | -171 | -137 | -143 | -119 | -95 | -113 | -147 | -137 | -253 | -119 | -171 | -223 | -165 | -143 | 137 |
| $10^{-1}$ EHC-GAL | 0 | -134 | -116 | -186 | -165 | -159 | -143 | -98 | -143 | -159 | -180 | -256 | -171 | -217 | -256 | -186 | -165 | 110 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -15 | -143 | -116 | -122 | -174 | -159 | -70 | -165 | -159 | -67 | -174 | -186 | -177 | -229 | -229 | -192 | 70 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -9 | -137 | -113 | -110 | -165 | -159 | -64 | -146 | -143 | -67 | -137 | -174 | -171 | -217 | -201 | -174 | 76 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -131 | -134 | -131 | -134 | -134 | -137 | -98 | -107 | -150 | -137 | -217 | -125 | -174 | -211 | -168 | -140 | 119 |
| $10^{-1}$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -137 | -149 | -171 | -174 | -171 | -155 | -125 | -134 | -171 | -165 | -250 | -168 | -216 | -235 | -195 | -177 | 46 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | -171 | -116 | -150 | -190 | -181 | -74 | -168 | -162 | -80 | -147 | -199 | -205 | -257 | -266 | -239 | 140 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 13 | -146 | -128 | -97 | -161 | -161 | -82 | -152 | -140 | -33 | -116 | -165 | -226 | -235 | -247 | -189 | 162 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -155 | -146 | -155 | -146 | -152 | -131 | -103 | -106 | -164 | -158 | -262 | -149 | -180 | -232 | -177 | -161 | 165 |
| $10^{-1}$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -137 | -122 | -171 | -128 | -125 | -107 | -70 | -85 | -122 | -119 | -223 | -88 | -158 | -195 | -146 | -122 | 183 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -241 | -458 | -406 | -369 | -445 | -449 | -256 | -403 | -378 | -223 | -323 | -415 | -427 | -488 | -455 | -394 | 415 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -262 | -470 | -427 | -378 | -466 | -451 | -259 | -418 | -378 | -241 | -341 | -399 | -418 | -485 | -457 | -375 | 327 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -412 | -357 | -403 | -363 | -354 | -320 | -235 | -247 | -290 | -269 | -458 | 46 | -92 | -165 | -67 | -46 | 760 |
| $10^{-1}$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -409 | -376 | -427 | -351 | -376 | -339 | -238 | -253 | -302 | -269 | -492 | -257 | -339 | -391 | -299 | -260 | 458 |
| $10^{-1}$ control | 0 | -36 | -73 | -82 | -73 | -85 | -85 | -39 | -58 | -51 | -30 | -54 | -82 | -61 | -82 | -85 | -73 | 177 |
| $10^{-1}$ control | 0 | -28 | -92 | -64 | -73 | -83 | -80 | -28 | -67 | -61 | -12 | -52 | -80 | -73 | -92 | -101 | -67 | 223 |
| $10^{-1}$ control | 0 | -92 | -92 | -95 | -80 | -71 | -62 | -74 | -65 | -86 | -71 | -113 | -62 | -83 | -104 | -65 | -49 | 183 |
| $10^{-1}$ control | 0 | -67 | -64 | -77 | -64 | -67 | -61 | -43 | -61 | -67 | -46 | -104 | -49 | -64 | -89 | -43 | -28 | 210 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| **C. freundii NCTC 9750** | **0** | **60** | **120** | **180** | **240** | **270** | **300** | **330** | **360** | **390** | **420** | **450** | **480** | **510** | **540** | **570** | **600** | **24h** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 4-MU-GAL | 0 | -80 | -156 | -113 | -119 | -159 | -156 | -89 | -147 | -107 | -101 | -135 | -162 | -125 | -165 | -168 | -159 | 140 |
| 1 4-MU-GAL | 0 | -76 | -143 | -119 | -125 | -146 | -152 | -88 | -134 | -143 | -106 | -128 | -137 | -149 | -161 | -174 | -155 | 101 |
| 1 4-MU-GAL | 0 | -119 | -128 | -134 | -137 | -110 | -122 | -94 | -100 | -128 | -94 | -168 | -107 | -137 | -146 | -110 | -82 | 165 |
| 1 4-MU-GAL | 0 | -125 | -128 | -152 | -122 | -119 | -125 | -91 | -116 | -113 | -116 | -174 | -101 | -107 | -131 | -97 | -107 | 171 |
| 1 EHC-GAL | 0 | -3 | -134 | -116 | -143 | -192 | -177 | -82 | -155 | -168 | -88 | -149 | -204 | -186 | -250 | -265 | -220 | 125 |
| 1 EHC-GAL | 0 | 28 | -6 | -12 | -6 | -3 | -3 | -12 | -12 | -3 | 10 | -9 | -15 | 0 | -15 | -9 | -15 | 10 |
| 1 EHC-GAL | 0 | -98 | -125 | -171 | -131 | -156 | -119 | -91 | -107 | -162 | -143 | -247 | -137 | -183 | -232 | -165 | -146 | 171 |
| 1 EHC-GAL | 0 | -137 | -140 | -210 | -161 | -155 | -161 | -100 | -128 | -161 | -161 | -265 | -164 | -213 | -256 | -189 | -170 | 129 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -39 | -149 | -116 | -143 | -165 | -158 | -85 | -146 | -149 | -64 | -140 | -186 | -183 | -219 | -241 | -189 | 92 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -9 | -138 | -125 | -116 | -150 | -159 | -86 | -135 | -138 | -73 | -138 | -138 | -177 | -214 | -196 | -168 | 88 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -128 | -125 | -165 | -125 | -137 | -128 | -110 | -113 | -137 | -134 | -217 | -128 | -174 | -204 | -159 | -143 | 171 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -107 | -122 | -153 | -116 | -122 | -129 | -77 | -104 | -144 | -113 | -196 | -119 | -156 | -183 | -132 | -147 | 125 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -6 | -171 | -165 | -135 | -217 | -202 | -89 | -180 | -187 | -89 | -156 | -202 | -217 | -269 | -278 | -272 | 128 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 9 | -147 | -135 | -104 | -171 | -184 | -68 | -165 | -153 | -74 | -144 | -193 | -196 | -263 | -275 | -217 | 143 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -149 | -143 | -183 | -177 | -152 | -149 | -109 | -116 | -155 | -143 | -250 | -149 | -207 | -238 | -174 | -155 | 51952 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -156 | -156 | -217 | -159 | -162 | -165 | -89 | -129 | -165 | -144 | -278 | -144 | -202 | -226 | -156 | -171 | 161 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -247 | -461 | -384 | -378 | -485 | -454 | -265 | -415 | -403 | -229 | -339 | -418 | -424 | -500 | -494 | -415 | 382 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -278 | -507 | -433 | -397 | -476 | -461 | -305 | -430 | -406 | -284 | -360 | -440 | -464 | -525 | -510 | -452 | 305 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -391 | -348 | -400 | -333 | -348 | -330 | -193 | -232 | -296 | -257 | -452 | 27 | -247 | -318 | -232 | -196 | 592 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -396 | -372 | -430 | -360 | -360 | -335 | -216 | -253 | -293 | -283 | -479 | -238 | -332 | -375 | -271 | -274 | 452 |
| 1control | 0 | -52 | -95 | -89 | -86 | -113 | -89 | -52 | -95 | -55 | -55 | -61 | -74 | -95 | -101 | -104 | -89 | 149 |
| 1control | 0 | -46 | -95 | -89 | -73 | -95 | -79 | -34 | -95 | -67 | -31 | -67 | -67 | -85 | -113 | -113 | -85 | 192 |
| 1control | 0 | -61 | -76 | -67 | -49 | -52 | -61 | -33 | -43 | -67 | -33 | -94 | -36 | -49 | -79 | -49 | -27 | 205 |
| 1control | 0 | -77 | -68 | -74 | -61 | -65 | -58 | -49 | -31 | -58 | -43 | -92 | -31 | -52 | -74 | -52 | -22 | 232 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| C. freundii NCTC 9750 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 4-MU-GAL | 0 | -64 | -147 | -129 | -135 | -144 | -168 | -80 | -132 | -138 | -98 | -110 | -135 | -135 | -171 | -171 | -150 | 168 |
| 10 4-MU-GAL | 0 | -64 | -137 | -122 | -116 | -149 | -137 | -85 | -134 | -140 | -67 | -125 | -134 | -128 | -167 | -158 | -116 | 37198 |
| 10 4-MU-GAL | 0 | -126 | -101 | -123 | -129 | -126 | -110 | -92 | -101 | -119 | -123 | -180 | -116 | -141 | -147 | -129 | -86 | 170 |
| 10 4-MU-GAL | 0 | -137 | -109 | -152 | -140 | -109 | -134 | -106 | -109 | -131 | -125 | -180 | -125 | -137 | -149 | -103 | -94 | 144 |
| 10 EHC-GAL | 0 | -22 | -171 | -153 | -147 | -199 | -187 | -104 | -168 | -168 | -95 | -183 | -205 | -202 | -254 | -248 | -159 | 51813 |
| 10 EHC-GAL | 0 | -3 | 0 | -6 | -3 | -6 | 7 | -21 | -6 | -9 | -6 | 10 | 0 | -3 | -18 | -6 | 4 | 28 |
| 10 EHC-GAL | 0 | -140 | -113 | -137 | -134 | -116 | -125 | -88 | -82 | -113 | -107 | -204 | -85 | -119 | -192 | -168 | -146 | 223 |
| 10 EHC-GAL | 0 | -147 | -159 | -202 | -174 | -165 | -171 | -116 | -122 | -171 | -183 | -281 | -168 | -205 | -257 | -186 | -174 | 125 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -9 | -119 | -125 | -122 | -155 | -155 | -73 | -140 | -140 | -79 | -137 | -165 | -162 | -210 | -235 | -183 | 98 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -46 | -146 | -155 | -149 | -180 | -183 | -107 | -155 | -158 | -70 | -152 | -204 | -180 | -235 | -216 | -192 | 95 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -89 | -119 | -119 | -110 | -128 | -119 | -89 | -98 | -113 | -107 | -202 | -104 | -138 | -174 | -144 | -131 | 131 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -140 | -134 | -153 | -125 | -131 | -122 | -95 | -110 | -137 | -134 | -223 | -98 | -143 | -195 | -125 | -110 | 42511 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -9 | -162 | -141 | -131 | -220 | -171 | -98 | -205 | -177 | -95 | -153 | -217 | -208 | -263 | -257 | -220 | 183 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -12 | -168 | -150 | -143 | -195 | -189 | -104 | -186 | -186 | -95 | -168 | -220 | -217 | -266 | -278 | -226 | 150 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -152 | -137 | -158 | -143 | -155 | -152 | -91 | -106 | -143 | -158 | -253 | -149 | -189 | -225 | -152 | -158 | 187 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -140 | -122 | -177 | -128 | -137 | -125 | -88 | -119 | -122 | -137 | -250 | -125 | -183 | -238 | -174 | -131 | 187 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -278 | -467 | -416 | -422 | -483 | -458 | -306 | -412 | -403 | -269 | -409 | -434 | -446 | -498 | -507 | -440 | 393 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -312 | -531 | -479 | -446 | -513 | -507 | -367 | -492 | -476 | -339 | -434 | -489 | -510 | -577 | -534 | -464 | 375 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -394 | -375 | -400 | -320 | -348 | -329 | -198 | -238 | -271 | -262 | -452 | 34 | -198 | -320 | -198 | -162 | 626 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -431 | -400 | -446 | -382 | -385 | -370 | -241 | -287 | -336 | -321 | -504 | -275 | -357 | -418 | -336 | -278 | 497 |
| 10control | 0 | -46 | -98 | -89 | -77 | -86 | -92 | -49 | -86 | -89 | -16 | -74 | -92 | -77 | -110 | -98 | -71 | 177 |
| 10control | 0 | -43 | -101 | -91 | -73 | -88 | -88 | -37 | -88 | -79 | -40 | -82 | -73 | -82 | -119 | -101 | -73 | 186 |
| 10control | 0 | -91 | -94 | -70 | -76 | -76 | -67 | -39 | -70 | -67 | -67 | -106 | -51 | -61 | -97 | -64 | -30 | 214 |
| 10control | 0 | -76 | -79 | -89 | -86 | -58 | -76 | -52 | -49 | -70 | -73 | -110 | -37 | -46 | -58 | -61 | -9 | 244 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| C. freundii NCTC 9750 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^2$ 4-MU-GAL | 0 | -77 | -153 | -144 | -147 | -162 | -159 | -89 | -144 | -144 | -95 | -119 | -144 | -144 | -153 | -147 | -110 | 39520 |
| $10^2$ 4-MU-GAL | 0 | -89 | -147 | -141 | -147 | -153 | -156 | -123 | -138 | -132 | -104 | -132 | -156 | -147 | -156 | -129 | -95 | 38162 |
| $10^2$ 4-MU-GAL | 0 | -131 | -128 | -137 | -137 | -137 | -122 | -109 | -119 | -128 | -125 | -161 | -94 | -131 | -91 | -36 | 80 | 39649 |
| $10^2$ 4-MU-GAL | 0 | -137 | -137 | -140 | -140 | -140 | -149 | -106 | -116 | -140 | -109 | -180 | -128 | -122 | -149 | -79 | -27 | 36856 |
| $10^2$ EHC-GAL | 0 | -37 | -196 | -162 | -153 | -211 | -199 | -132 | -193 | -196 | -116 | -171 | -226 | -229 | -275 | -284 | -208 | 51810 |
| $10^2$ EHC-GAL | 0 | 7 | 10 | -21 | -3 | 3 | 0 | 13 | 10 | 3 | 3 | 0 | 10 | 3 | -9 | -12 | 7 | 55 |
| $10^2$ EHC-GAL | 0 | -162 | -147 | -177 | -162 | -137 | -153 | -101 | -128 | -143 | -137 | -244 | -137 | -177 | -186 | -40 | 128 | 50999 |
| $10^2$ EHC-GAL | 0 | -143 | -158 | -192 | -167 | -152 | -173 | -134 | -128 | -158 | -161 | -277 | -146 | -192 | -225 | -122 | -54 | 51265 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -31 | -144 | -122 | -104 | -159 | -153 | -89 | -137 | -137 | -86 | -150 | -183 | -162 | -202 | -214 | -162 | 43210 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -33 | -161 | -146 | -119 | -192 | -174 | -97 | -161 | -155 | -116 | -152 | -180 | -180 | -210 | -201 | -122 | 43623 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -135 | -144 | -150 | -113 | -132 | -125 | -74 | -101 | -132 | -128 | -223 | -122 | -138 | -183 | -119 | -70 | 43073 |
| $10^2$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -134 | -128 | -146 | -140 | -140 | -125 | -88 | -110 | -137 | -113 | -198 | -119 | -162 | -201 | -153 | -101 | 42893 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -12 | -168 | -134 | -143 | -208 | -198 | -92 | -189 | -174 | -92 | -174 | -220 | -226 | -262 | -262 | -204 | 53175 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -21 | -180 | -144 | -125 | -198 | -211 | -89 | -183 | -162 | -116 | -150 | -189 | -214 | -256 | -266 | -159 | 53404 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -143 | -122 | -180 | -137 | -143 | -134 | -73 | -94 | -134 | -131 | -250 | -116 | -174 | -198 | -67 | 64 | 52550 |
| $10^2$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -161 | -143 | -174 | -149 | -131 | -131 | -79 | -100 | -152 | -131 | -253 | -131 | -180 | -232 | -161 | -146 | 229 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -266 | -476 | -433 | -400 | -510 | -446 | -296 | -400 | -400 | -269 | -351 | -424 | -440 | -498 | -470 | -391 | 52265 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -345 | -610 | -580 | -534 | -632 | -610 | -445 | -583 | -567 | -448 | -528 | -589 | -604 | -668 | -647 | -580 | 51524 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -403 | -354 | -361 | -321 | -312 | -300 | -184 | -223 | -284 | -223 | -437 | -10 | -141 | -235 | -119 | -55 | 48203 |
| $10^2$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -437 | -388 | -409 | -339 | -367 | -357 | -235 | -266 | -312 | -284 | -492 | -260 | -333 | -424 | -296 | -238 | 48057 |
| $10^2$ control | 0 | -33 | -79 | -70 | -73 | -82 | -67 | -36 | -67 | -73 | -36 | -67 | -82 | -70 | -79 | -106 | -58 | 205 |
| $10^2$ control | 0 | -28 | -98 | -64 | -61 | -76 | -89 | -40 | -70 | -76 | -31 | -64 | -64 | -79 | -92 | -107 | -89 | 180 |
| $10^2$ control | 0 | -94 | -64 | -64 | -73 | -55 | -58 | -42 | -52 | -64 | -45 | -91 | -33 | -58 | -73 | -52 | -18 | 220 |
| $10^2$ control | 0 | -77 | -77 | -83 | -61 | -77 | -46 | -49 | -43 | -55 | -61 | -95 | -49 | -67 | -74 | -77 | -25 | 244 |

## Appendix 5.1 (Cont'd..): Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli FRHECO2, K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| **C. freundii NCTC 9750** | **0** | **60** | **120** | **180** | **240** | **270** | **300** | **330** | **360** | **390** | **420** | **450** | **480** | **510** | **540** | **570** | **600** | **24h** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^3$ 4-MU-GAL | 0 | -76 | -147 | -141 | -122 | -165 | -180 | -92 | -141 | -131 | -76 | -95 | -58 | 55 | 622 | 2072 | 4447 | 43094 |
| $10^3$ 4-MU-GAL | 0 | -89 | -159 | -165 | -141 | -150 | -150 | -101 | -141 | -150 | -110 | -125 | -101 | -40 | 140 | 857 | 2393 | 41132 |
| $10^3$ 4-MU-GAL | 0 | -128 | -113 | -128 | -119 | -119 | -107 | -94 | -94 | -100 | -52 | -137 | -3 | 89 | 583 | 2607 | 5561 | 42893 |
| $10^3$ 4-MU-GAL | 0 | -144 | -138 | -126 | -141 | -129 | -138 | -113 | -107 | -132 | -113 | -187 | -89 | -55 | 33 | 418 | 1199 | 39477 |
| $10^3$ EHC-GAL | 0 | -16 | -165 | -153 | -122 | -177 | -205 | -107 | -174 | -171 | -86 | -144 | -156 | -74 | 128 | 949 | 2679 | 53202 |
| $10^3$ EHC-GAL | 0 | 7 | 0 | 3 | 7 | -3 | 3 | 13 | 10 | 16 | 13 | 10 | -3 | -3 | 3 | 7 | 0 | 89 |
| $10^3$ EHC-GAL | 0 | -177 | -150 | -165 | -186 | -180 | -177 | -128 | -141 | -168 | -156 | -275 | -128 | -150 | -70 | 332 | 937 | 52528 |
| $10^3$ EHC-GAL | 0 | -162 | -153 | -180 | -183 | -162 | -174 | -122 | -125 | -171 | -150 | -256 | -113 | -95 | 107 | 925 | 2118 | 53074 |
| $10^3$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -39 | -131 | -116 | -122 | -158 | -201 | -91 | -164 | -170 | -103 | -140 | -170 | -155 | -131 | -3 | 489 | 45274 |
| $10^3$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -30 | -131 | -125 | -122 | -171 | -177 | -85 | -143 | -158 | -85 | -140 | -161 | -122 | -91 | 138 | 620 | 45252 |
| $10^3$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -147 | -178 | -168 | -153 | -171 | -159 | -107 | -129 | -150 | -162 | -232 | -132 | -141 | -98 | 210 | 772 | 44754 |
| $10^3$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -122 | -137 | -150 | -125 | -104 | -125 | -104 | -89 | -134 | -89 | -195 | -110 | -110 | -82 | 168 | 559 | 44828 |
| $10^3$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -24 | -168 | -152 | -143 | -192 | -195 | -91 | -174 | -171 | -88 | -156 | -192 | -162 | -76 | 305 | 1190 | 54451 |
| $10^3$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -12 | -162 | -125 | -137 | -186 | -186 | -91 | -174 | -177 | -76 | -140 | -174 | -119 | -6 | 482 | 1563 | 54503 |
| $10^3$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -140 | -152 | -164 | -140 | -161 | -140 | -88 | -103 | -131 | -134 | -250 | -100 | -106 | 80 | 831 | 1981 | 53417 |
| $10^3$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -141 | -135 | -165 | -156 | -144 | -147 | -92 | -110 | -150 | -119 | -260 | -95 | -80 | 140 | 1065 | 2484 | 53516 |
| $10^3$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -277 | -485 | -457 | -418 | -506 | -482 | -323 | -457 | -442 | -296 | -384 | -451 | -433 | -457 | -369 | 68 | 53502 |
| $10^3$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -363 | -623 | -565 | -537 | -632 | -623 | -473 | -577 | -574 | -418 | -559 | -574 | -580 | -556 | -290 | 259 | 53306 |
| $10^3$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -412 | -397 | -397 | -342 | -354 | -333 | -235 | -238 | -309 | -251 | -458 | -55 | -214 | -241 | 171 | 741 | 49448 |
| $10^3$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -436 | -403 | -412 | -354 | -363 | -336 | -253 | -247 | -314 | -278 | -476 | -256 | -332 | -360 | -94 | 229 | 49598 |
| $10^3$ control | 0 | -52 | -113 | -89 | -83 | -104 | -101 | -58 | -76 | -95 | -61 | -70 | -98 | -67 | -125 | -116 | -86 | 217 |
| $10^3$ control | 0 | -40 | -89 | -79 | -70 | -107 | -95 | -28 | -67 | -61 | -37 | -79 | -76 | -67 | -98 | -89 | -67 | 195 |
| $10^3$ control | 0 | -77 | -80 | -77 | -86 | -55 | -43 | -49 | -46 | -74 | -65 | -107 | -49 | -58 | -86 | -49 | -37 | 253 |
| $10^3$ control | 0 | -95 | -95 | -104 | -95 | -85 | -95 | -67 | -67 | -76 | -88 | -119 | -73 | -95 | -92 | -58 | -40 | 247 |

## Appendix 5.1: Zeroed fluorescence data (4 replicates) of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| C. freundii NCTC 9750 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^4$ 4-MU-GAL | 0 | -83 | -153 | -147 | -116 | -165 | -153 | -110 | -110 | -67 | 116 | 503 | 1950 | 4508 | 11097 | 18245 | 24013 | 44126 |
| $10^4$ 4-MU-GAL | 0 | -70 | -159 | -147 | -147 | -150 | -162 | -89 | -144 | -92 | 9 | 82 | 604 | 1758 | 5860 | 11775 | 17543 | 42835 |
| $10^4$ 4-MU-GAL | 0 | -165 | -159 | -168 | -165 | -147 | -153 | -125 | -116 | -98 | 430 | -18 | 1730 | 3968 | 10407 | 19572 | 24920 | 43305 |
| $10^4$ 4-MU-GAL | 0 | -147 | -129 | -135 | -132 | -144 | -138 | -119 | -110 | -110 | 52 | -89 | 457 | 1373 | 5521 | 12461 | 18333 | 40915 |
| $10^4$ EHC-GAL | 0 | -15 | -165 | -137 | -131 | -192 | -195 | -95 | -162 | -137 | -3 | 67 | 473 | 1550 | 5423 | 11936 | 17515 | 54149 |
| $10^4$ EHC-GAL | 0 | 10 | -6 | 22 | 10 | -9 | 3 | 10 | -3 | 7 | 0 | 7 | 10 | 3 | 3 | 10 | 13 | 55 |
| $10^4$ EHC-GAL | 0 | -149 | -137 | -161 | -152 | -158 | -158 | -116 | -97 | -106 | 77 | -158 | 443 | 1426 | 5967 | 13347 | 19790 | 52312 |
| $10^4$ EHC-GAL | 0 | -150 | -150 | -183 | -186 | -180 | -186 | -147 | -153 | -159 | 107 | -183 | 861 | 2362 | 7822 | 16572 | 23497 | 51698 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -30 | -152 | -113 | -119 | -171 | -155 | -55 | -149 | -140 | -49 | -79 | 52 | 428 | 2018 | 5073 | 8784 | 46424 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -19 | -165 | -135 | -147 | -181 | -171 | -104 | -171 | -144 | -71 | -65 | 39 | 421 | 1999 | 5048 | 8652 | 45700 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -122 | -116 | -140 | -128 | -131 | -134 | -91 | -110 | -125 | -36 | -192 | 28 | 281 | 1783 | 4972 | 9110 | 46223 |
| $10^4$ 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | -122 | -104 | -125 | -116 | -122 | -125 | -83 | -107 | -113 | -37 | -165 | 177 | 595 | 2921 | 7492 | 12794 | 44995 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -28 | -168 | -134 | -140 | -180 | -202 | -76 | -171 | -147 | 3 | 37 | 534 | 1654 | 5353 | 11219 | 15883 | 54997 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -6 | -198 | -143 | -156 | -195 | -198 | -91 | -192 | -156 | -27 | 12 | 351 | 1163 | 4364 | 9657 | 14335 | 54570 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -143 | -137 | -161 | -128 | -164 | -158 | -94 | -100 | -128 | 31 | -192 | 309 | 1032 | 4661 | 10801 | 16634 | 53514 |
| $10^4$ methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | -141 | -138 | -171 | -159 | -171 | -168 | -98 | -123 | -141 | 24 | -196 | 521 | 1413 | 5603 | 13016 | 19780 | 52735 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -263 | -467 | -440 | -394 | -498 | -443 | -303 | -416 | -397 | -229 | -333 | -260 | -49 | 1114 | 3372 | 6049 | 53892 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -345 | -638 | -583 | -577 | -684 | -659 | -498 | -641 | -607 | -406 | -449 | -128 | 565 | 3256 | 7685 | 11237 | 53697 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -394 | -370 | -400 | -354 | -354 | -309 | -232 | -244 | -275 | -193 | -431 | 107 | 299 | 2069 | 5792 | 9482 | 50245 |
| $10^4$ 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | -418 | -394 | -406 | -348 | -360 | -354 | -241 | -272 | -278 | -153 | -431 | 198 | 885 | 3857 | 8851 | 12467 | 49409 |
| $10^4$ control | 0 | -34 | -92 | -74 | -83 | -95 | -86 | -37 | -70 | -77 | -31 | -58 | -83 | -61 | -101 | -101 | -70 | 235 |
| $10^4$ control | 0 | -25 | -71 | -71 | -58 | -80 | -77 | -37 | -58 | -67 | -28 | -40 | -71 | -77 | -89 | -95 | -64 | 201 |
| $10^4$ control | 0 | -98 | -92 | -80 | -101 | -70 | -89 | -61 | -86 | -70 | -70 | -110 | -52 | -77 | -89 | -70 | -31 | 265 |
| $10^4$ control | 0 | -68 | -65 | -65 | -52 | -55 | -65 | -43 | -46 | -55 | -62 | -101 | -49 | -68 | -89 | -40 | -7 | 228 |

## Appendix 5.2: Pattern of positive/negative wells of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.

| *E. coli* FRHECO2 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10000 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 1000 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 4 | 4 | 4 | 4 | 4 | 4 |
| 100 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 4 | 4 | 4 |
| 10 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-MU-GAL MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 23 | 112.6 | 231.2 | 361.7 | 1145.4 | 2399.7 | 2399.7 | 2399.7 |
| 10000 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 1000 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 100 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 4 | 4 | 4 | 4 |
| 10 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| 1 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| EHC-GAL MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 6 | 61.5 | 112.6 | 231.2 | 621.7 | 1145.4 | 3852.9 | 3852.9 | 3852.9 | 3852.9 |
| 10000 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 1000 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 4 | 4 | 4 | 4 | 4 | 4 |
| 100 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 4 | 4 | 4 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9.3 | 36 | 61.5 | 231.2 | 621.7 | 3852.9 | 3852.9 | 3852.9 | 3852.9 |
| 10000 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 1000 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 4 | 4 | 4 | 4 | 4 | 4 |
| 100 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 3 | 3 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 2 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 6 | 36 | 61.5 | 61.5 | 231.2 | 621.7 | 939.7 | 2184.1 | 2184.1 | 2184.1 |
| 10000 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 1000 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 4 | 4 | 4 | 4 |
| 100 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 4 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.5 | 23 | 23 | 61.5 | 231.2 | 231.2 | 621.7 | 1626.2 | 1626.2 |

**Appendix 5.2 (Cont'd..): Pattern of positive/negative wells of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.**

| *K. pneumoniae* NCTC 10896 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10000 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 1000 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 100 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 |
| 10 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| 1 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-MU-GAL MPN | 0 | 0 | 0 | 0 | 0 | 0 | 9.2 | 23.1 | 23.1 | 93.3 | 239.8 | 239.8 | 462.3 | 2310.5 | 4274 | 4274 | 4274 | 4274 |
| 10000 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 1000 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 100 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 3 | 3 |
| 10 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 |
| 1 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| EHC-GAL MPN | 0 | 0 | 0 | 0 | 0 | 9.2 | 23.1 | 23.1 | 23.1 | 239.8 | 239.8 | 1099.4 | 2310.5 | 2310.5 | 2310.5 | 9366.1 | 24282.73 | 24282.73 |
| 10000 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 1000 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 100 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 2 | 3 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23.1 | 23.1 | 93.3 | 239.8 | 239.8 | 462.3 | 4274 | 9366.1 | 9366.1 | 9366.1 | 24282.73 |
| 10000 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 1000 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 100 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside MPN | 0 | 0 | 0 | 0 | 0 | 3.6 | 9.2 | 23.1 | 23.1 | 239.8 | 239.8 | 239.8 | 462.3 | 2310.5 | 2310.5 | 4274 | 9366.1 | 9366.1 |
| 10000 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 1000 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 |
| 100 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 3 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23.1 | 23.1 | 23.1 | 239.8 | 239.8 | 462.3 | 462.3 | 2310.5 | 9366.1 |

**Appendix 5.2 (Cont'd..): Pattern of positive/negative wells of 5 fluorogenic ß-D-galactoside substrates with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750.**

| *C. freundii* NCTC 9750 | 0 | 60 | 120 | 180 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10000 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 4 | 4 | 4 | 4 | 4 | 4 |
| 1000 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 4 | 4 | 4 |
| 100 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| 10 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1 4-MU-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-MU-GAL MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 6 | 23 | 61.5 | 231.2 | 231.2 | 361.7 | 3852.9 |
| 10000 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 |
| 1000 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 |
| 100 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 |
| 10 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1 EHC-GAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| EHC-GAL MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.6 | 23.1 | 33.1 | 93.3 | 239.8 | 462.3 | 4274 |
| 10000 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 4 | 4 | 4 | 4 |
| 1000 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 4 | 4 |
| 100 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 10 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-hydroxycoumarin-3-carboxylic acid-ß-D-galactoside MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 23 | 23 | 112.6 | 231.2 | 3852.9 |
| 10000 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 4 | 4 | 4 | 4 | 4 | 4 |
| 1000 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 4 | 4 |
| 100 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 |
| 10 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| methyl 7-hydroxycoumarin-3-carboxylate-ß-D-galactoside MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.5 | 6 | 23 | 23 | 61.5 | 231.2 | 361.7 | 1969.6 |
| 10000 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 4 | 4 | 4 | 4 |
| 1000 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 4 |
| 100 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 10 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-chloro-7-hydroxy-4-methylcoumarin-ß-D-galactoside MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 11.3 | 23 | 36 | 231.2 | 2399.7 |

**Appendix 5.3: Pattern of positive/negative wells in LTB with *E. coli* FRHECO2, *K. pneumoniae* NCTC 10896 and *C. freundii* NCTC 9750 at 24 h and 48 h.**

| ***E. coli* FRHECO2** | **24h** | **48h** |
|---|---|---|
| 10000 LTB | 4 | 4 |
| 1000 LTB | 4 | 4 |
| 100 LTB | 3 | 3 |
| 10 LTB | 0 | 0 |
| 1 LTB | 0 | 0 |
| LTB MPN | 1969.6 | 1969.6 |

| ***K. pneumoniae* NCTC 10896** | **24h** | **48h** |
|---|---|---|
| 10000 LTB | 4 | 4 |
| 1000 LTB | 4 | 4 |
| 100 LTB | 4 | 4 |
| 10 LTB | 3 | 3 |
| 1 LTB | 0 | 0 |
| LTB MPN | 11493 | 11493 |

| ***C. freundii* NCTC 9750** | **24h** | **48h** |
|---|---|---|
| 10000 LTB | 4 | 4 |
| 1000 LTB | 4 | 4 |
| 100 LTB | 2 | 2 |
| 10 LTB | 0 | 0 |
| 1 LTB | 0 | 0 |
| LTB MPN | 621.7 | 621.7 |

## Appendix 5.4: Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*C. freundii* NCTC 9750 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -415 | -235 | -287 | -290 | -296 | -275 | -299 | -326 | -317 | -323 | -360 | -278 |
| 20 | 2 | 0 | -399 | -238 | -271 | -274 | -274 | -259 | -277 | -250 | -198 | 180 | 1383 | 58159 |
| 20 | 3 | 0 | -464 | -251 | -336 | -324 | -293 | -348 | -351 | -248 | -138 | 512 | 1767 | 57524 |
| 20 | 4 | 0 | -430 | -189 | -265 | -274 | -207 | -256 | -284 | -122 | -6 | 547 | 1548 | 56920 |
| 20 | 5 | 0 | -480 | -263 | -339 | -315 | -339 | -385 | -379 | -382 | -394 | -403 | -385 | 54224 |
| 20 | 6 | 0 | -489 | -278 | -351 | -388 | -357 | -412 | -394 | -409 | -385 | -385 | -330 | 52815 |
| 20 | 7 | 0 | -522 | -339 | -385 | -412 | -354 | -418 | -437 | -446 | -400 | -364 | -217 | 49460 |
| 20 | 8 | 0 | -491 | -320 | -384 | -387 | -397 | -448 | -448 | -473 | -455 | -323 | -128 | 47511 |
| 20 | 9 | 0 | -400 | -220 | -296 | -278 | -284 | -250 | -268 | -207 | -143 | 272 | 1325 | 58876 |
| 20 | 10 | 0 | -427 | -256 | -283 | -299 | -283 | -277 | -305 | -296 | -280 | -213 | -18 | 58236 |
| 20 | 11 | 0 | -488 | -266 | -345 | -351 | -311 | -354 | -366 | -366 | -372 | -400 | -427 | -372 |
| 20 | 12 | 0 | -457 | -210 | -259 | -265 | -222 | -277 | -305 | -308 | -384 | -351 | -357 | -299 |
| 20 | 13 | 0 | -513 | -269 | -342 | -345 | -308 | -357 | -366 | -388 | -409 | -415 | -403 | -345 |
| 20 | 14 | 0 | -431 | -220 | -281 | -269 | -275 | -311 | -235 | -119 | 79 | 360 | 864 | 55000 |
| 20 | 15 | 0 | -480 | -287 | -321 | -339 | -306 | -336 | -351 | -361 | -324 | -275 | -199 | 53107 |
| 20 | 16 | 0 | -491 | -274 | -342 | -317 | -342 | -342 | -369 | -390 | -381 | -357 | -268 | 51841 |
| 20 | 17 | 0 | -454 | -253 | -268 | -283 | -286 | -286 | -311 | -326 | -332 | -311 | -369 | 57149 |
| 20 | 18 | 0 | -409 | -272 | -266 | -247 | -220 | -244 | -284 | -296 | -305 | -321 | -339 | -260 |
| 20 | 19 | 0 | -486 | -248 | -324 | -303 | -303 | -342 | -339 | -351 | -333 | -364 | -379 | -336 |
| 20 | 20 | 0 | -428 | -177 | -229 | -241 | -217 | -244 | -269 | -284 | -302 | -321 | -327 | -281 |
| 20 | 21 | 0 | -452 | -226 | -299 | -262 | -290 | -302 | -305 | -311 | -278 | -192 | 128 | 56688 |
| 20 | 22 | 0 | -433 | -223 | -275 | -269 | -290 | -326 | -308 | -354 | -333 | -351 | -363 | -299 |
| 20 | 23 | 0 | -494 | -302 | -317 | -317 | -244 | -332 | -302 | -204 | -97 | 275 | 748 | 54619 |
| 20 | 24 | 0 | -446 | -250 | -302 | -314 | -272 | -336 | -342 | -375 | -394 | -381 | -436 | -369 |
| 20 | 25 | 0 | -492 | -275 | -309 | -327 | -299 | -312 | -318 | -321 | -345 | -248 | -147 | 58162 |
| 20 | 26 | 0 | -409 | -275 | -272 | -275 | -229 | -232 | -293 | -339 | -317 | -321 | -317 | -253 |
| 20 | 27 | 0 | -467 | -248 | -321 | -287 | -309 | -336 | -318 | -269 | -144 | 100 | 1129 | 58143 |
| 20 | 28 | 0 | -430 | -180 | -229 | -235 | -202 | -217 | -220 | 27 | 336 | 1486 | 3595 | 58226 |
| 20 | 29 | 0 | -446 | -223 | -278 | -253 | -250 | -272 | -275 | -287 | -247 | -137 | 140 | 57008 |
| 20 | 30 | 0 | -443 | -223 | -308 | -284 | -263 | -324 | -275 | -290 | -208 | 30 | 702 | 56715 |
| 20 | 31 | 0 | -467 | -268 | -311 | -290 | -296 | -308 | -308 | -284 | -226 | -27 | 260 | 55009 |
| 20 | 32 | 0 | -422 | -235 | -266 | -293 | -232 | -272 | -251 | -165 | -34 | 332 | 882 | 55290 |
| 10 | 1 | 0 | -406 | -266 | -260 | -257 | -260 | -248 | -309 | -275 | -281 | -132 | 110 | 58018 |
| 10 | 2 | 0 | -433 | -293 | -284 | -275 | -259 | -265 | -311 | -354 | -339 | -329 | -363 | -317 |
| 10 | 3 | 0 | -455 | -223 | -281 | -272 | -287 | -293 | -303 | -312 | -303 | -242 | -257 | -196 |
| 10 | 4 | 0 | -434 | -174 | -248 | -241 | -220 | -241 | -248 | -278 | -251 | -217 | -104 | 57728 |
| 10 | 5 | 0 | -443 | -186 | -269 | -244 | -260 | -275 | -266 | -278 | -214 | 24 | 476 | 57191 |
| 10 | 6 | 0 | -430 | -207 | -265 | -250 | -244 | -287 | -272 | -299 | -290 | -302 | -326 | -238 |
| 10 | 7 | 0 | -464 | -271 | -308 | -293 | -271 | -323 | -293 | -274 | -235 | 49 | 675 | 55367 |
| 10 | 8 | 0 | -443 | -244 | -290 | -278 | -254 | -290 | -296 | -363 | -348 | -315 | -388 | -336 |
| 10 | 9 | 0 | -376 | -257 | -257 | -254 | -263 | -251 | -290 | -303 | -300 | -318 | -336 | -281 |
| 10 | 10 | 0 | -439 | -305 | -275 | -281 | -241 | -256 | -299 | -320 | -314 | -332 | -354 | -314 |
| 10 | 11 | 0 | -476 | -232 | -299 | -299 | -266 | -327 | -327 | -323 | -308 | -345 | -381 | -345 |
| 10 | 12 | 0 | -470 | -211 | -287 | -299 | -266 | -296 | -305 | -320 | -327 | -351 | -369 | -269 |
| 10 | 13 | 0 | -433 | -195 | -274 | -229 | -250 | -274 | -280 | -293 | -250 | -155 | 31 | 57103 |
| 10 | 14 | 0 | -412 | -201 | -271 | -253 | -238 | -299 | -259 | -287 | -296 | -305 | -302 | -271 |
| 10 | 15 | 0 | -467 | -263 | -275 | -293 | -263 | -305 | -287 | -150 | 37 | 818 | 2390 | 55397 |
| 10 | 16 | 0 | -427 | -235 | -265 | -247 | -250 | -296 | -296 | -348 | -335 | -329 | -348 | -317 |
| 10 | 17 | 0 | -385 | -241 | -287 | -260 | -260 | -266 | -290 | -333 | -315 | -296 | -327 | -318 |
| 10 | 18 | 0 | -433 | -293 | -281 | -272 | -290 | -265 | -314 | -308 | -311 | -323 | -351 | -326 |
| 10 | 19 | 0 | -458 | -214 | -308 | -302 | -299 | -302 | -324 | -318 | -327 | -327 | -357 | -314 |
| 10 | 20 | 0 | -479 | -201 | -275 | -293 | -238 | -275 | -311 | -351 | -320 | -348 | -354 | -305 |
| 10 | 21 | 0 | -470 | -208 | -254 | -247 | -263 | -315 | -290 | -336 | -330 | -305 | -339 | -299 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*C. freundii* NCTC 9750 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -449 | -226 | -299 | -275 | -257 | -290 | -269 | -312 | -305 | -296 | -321 | -299 |
| 10 | 23 | 0 | -482 | -293 | -308 | -320 | -284 | -326 | -329 | -326 | -342 | -339 | -378 | -354 |
| 10 | 24 | 0 | -412 | -223 | -271 | -271 | -262 | -275 | -278 | -345 | -336 | -308 | -348 | -314 |
| 10 | 25 | 0 | -384 | -204 | -241 | -265 | -241 | -235 | -277 | -311 | -311 | -311 | -296 | -289 |
| 10 | 26 | 0 | -427 | -269 | -293 | -266 | -302 | -259 | -311 | -314 | -342 | -339 | -333 | -323 |
| 10 | 27 | 0 | -421 | -189 | -263 | -272 | -281 | -272 | -284 | -296 | -308 | -308 | -345 | -275 |
| 10 | 28 | 0 | -412 | -161 | -226 | -247 | -213 | -250 | -262 | -280 | -250 | -241 | -161 | 57338 |
| 10 | 29 | 0 | -433 | -207 | -287 | -238 | -256 | -308 | -299 | -329 | -299 | -302 | -311 | -296 |
| 10 | 30 | 0 | -418 | -220 | -278 | -263 | -229 | -272 | -275 | -314 | -330 | -275 | -308 | -281 |
| 10 | 31 | 0 | -476 | -272 | -293 | -284 | -305 | -318 | -318 | -275 | -281 | -156 | 61 | 55461 |
| 10 | 32 | 0 | -415 | -226 | -290 | -293 | -277 | -265 | -268 | -308 | -259 | -195 | -91 | 55660 |
| 5 | 1 | 0 | -387 | -241 | -253 | -311 | -265 | -250 | -305 | -341 | -326 | -289 | -317 | -311 |
| 5 | 2 | 0 | -458 | -275 | -290 | -272 | -296 | -265 | -299 | -320 | -311 | -317 | -381 | -302 |
| 5 | 3 | 0 | -437 | -229 | -278 | -287 | -281 | -293 | -296 | -324 | -324 | -327 | -351 | -281 |
| 5 | 4 | 0 | -446 | -165 | -239 | -257 | -226 | -260 | -263 | -217 | -190 | 21 | 686 | 57713 |
| 5 | 5 | 0 | -451 | -195 | -271 | -250 | -280 | -323 | -286 | -347 | -341 | -311 | -332 | -286 |
| 5 | 6 | 0 | -461 | -242 | -290 | -272 | -242 | -306 | -296 | -293 | -324 | -306 | -336 | -342 |
| 5 | 7 | 0 | -479 | -284 | -327 | -296 | -302 | -354 | -342 | -339 | -366 | -345 | -369 | -382 |
| 5 | 8 | 0 | -436 | -259 | -287 | -281 | -275 | -259 | -305 | -305 | -357 | -354 | -357 | -320 |
| 5 | 9 | 0 | -422 | -245 | -278 | -278 | -287 | -251 | -309 | -302 | -306 | -312 | -324 | -278 |
| 5 | 10 | 0 | -436 | -265 | -293 | -296 | -275 | -272 | -314 | -333 | -323 | -317 | -363 | -320 |
| 5 | 11 | 0 | -437 | -248 | -290 | -284 | -272 | -275 | -306 | -315 | -312 | -306 | -342 | -306 |
| 5 | 12 | 0 | -467 | -226 | -296 | -305 | -274 | -342 | -326 | -338 | -354 | -354 | -378 | -320 |
| 5 | 13 | 0 | -458 | -220 | -287 | -293 | -275 | -299 | -318 | -354 | -354 | -330 | -324 | -345 |
| 5 | 14 | 0 | -461 | -275 | -308 | -290 | -290 | -317 | -330 | -293 | -321 | -339 | -363 | -360 |
| 5 | 15 | 0 | -470 | -296 | -330 | -296 | -263 | -345 | -327 | -330 | -342 | -339 | -363 | -382 |
| 5 | 16 | 0 | -449 | -293 | -308 | -311 | -327 | -296 | -339 | -366 | -376 | -369 | -421 | -357 |
| 5 | 17 | 0 | -376 | -251 | -251 | -260 | -290 | -238 | -281 | -266 | -205 | 30 | 412 | 57731 |
| 5 | 18 | 0 | -397 | -260 | -266 | -278 | -241 | -241 | -293 | -308 | -308 | -305 | -324 | -278 |
| 5 | 19 | 0 | -430 | -259 | -250 | -286 | -280 | -265 | -317 | -311 | -326 | -311 | -338 | -302 |
| 5 | 20 | 0 | -470 | -238 | -290 | -311 | -308 | -344 | -326 | -363 | -363 | -338 | -372 | -338 |
| 5 | 21 | 0 | -446 | -226 | -293 | -305 | -290 | -311 | -321 | -336 | -336 | -366 | -357 | -302 |
| 5 | 22 | 0 | -448 | -253 | -277 | -287 | -262 | -302 | -308 | -290 | -329 | -308 | -342 | -366 |
| 5 | 23 | 0 | -436 | -287 | -290 | -272 | -272 | -305 | -317 | -299 | -330 | -323 | -336 | -336 |
| 5 | 24 | 0 | -433 | -268 | -302 | -314 | -280 | -289 | -305 | -341 | -372 | -354 | -363 | -323 |
| 5 | 25 | 0 | -348 | -256 | -226 | -265 | -259 | -253 | -311 | -317 | -299 | -302 | -287 | -235 |
| 5 | 26 | 0 | -445 | -268 | -278 | -290 | -290 | -201 | -235 | -189 | -70 | 424 | 1160 | 58235 |
| 5 | 27 | 0 | -409 | -260 | -281 | -290 | -293 | -275 | -290 | -317 | -314 | -321 | -336 | -281 |
| 5 | 28 | 0 | -467 | -210 | -265 | -320 | -314 | -320 | -323 | -354 | -320 | -332 | -363 | -290 |
| 5 | 29 | 0 | -431 | -177 | -247 | -281 | -266 | -302 | -281 | -235 | -131 | 168 | 1031 | 58226 |
| 5 | 30 | 0 | -458 | -247 | -302 | -284 | -262 | -302 | -299 | -281 | -330 | -287 | -348 | -336 |
| 5 | 31 | 0 | -434 | -272 | -272 | -254 | -257 | -318 | -302 | -293 | -321 | -318 | -333 | -330 |
| 5 | 32 | 0 | -449 | -284 | -306 | -312 | -324 | -296 | -333 | -318 | -263 | -37 | 268 | 55103 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*K. pneumoniae* NCTC 10896 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | 58 | -82 | -189 | -204 | -293 | -67 | -146 | -281 | -369 | -330 | -427 | -174 |
| 20 | 2 | 0 | 208 | 403 | 1120 | 3620 | 6797 | 14586 | 21627 | 30703 | 32153 | 35523 | 34952 | 42860 |
| 20 | 3 | 0 | 101 | 97 | 244 | 1123 | 2368 | 5750 | 10770 | 18901 | 22505 | 29452 | 31393 | 41867 |
| 20 | 4 | 0 | 161 | 222 | 479 | 1602 | 3134 | 7617 | 14671 | 22923 | 25853 | 30956 | 32079 | 41424 |
| 20 | 5 | 0 | 95 | 61 | 91 | 476 | 946 | 2796 | 5374 | 10740 | 13841 | 20982 | 26104 | 40836 |
| 20 | 6 | 0 | 76 | 46 | 70 | 415 | 824 | 2466 | 4923 | 9925 | 13249 | 20162 | 25490 | 40766 |
| 20 | 7 | 0 | 119 | 125 | 235 | 894 | 1593 | 4053 | 7584 | 14240 | 17906 | 24467 | 27867 | 40277 |
| 20 | 8 | 0 | 73 | 12 | 24 | 320 | 601 | 1917 | 3729 | 7560 | 10300 | 16856 | 22298 | 40277 |
| 20 | 9 | 0 | 64 | -36 | -140 | -137 | -165 | 189 | 516 | 1535 | 2445 | 6644 | 11174 | 44663 |
| 20 | 10 | 0 | 89 | 6 | -42 | 251 | 888 | 3434 | 7578 | 16371 | 21102 | 32403 | 34775 | 44666 |
| 20 | 11 | 0 | 55 | -36 | -137 | -122 | -55 | 437 | 1172 | 3254 | 4957 | 10322 | 15950 | 43546 |
| 20 | 12 | 0 | 55 | -36 | -128 | -48 | 55 | 916 | 2500 | 6373 | 8915 | 15651 | 21584 | 44297 |
| 20 | 13 | 0 | 177 | 265 | 708 | 2655 | 5353 | 13480 | 24343 | 34494 | 34735 | 36447 | 36087 | 44065 |
| 20 | 14 | 0 | 131 | 165 | 421 | 1730 | 3525 | 9028 | 16783 | 26583 | 30184 | 34264 | 34875 | 44373 |
| 20 | 15 | 0 | 94 | 49 | 18 | 424 | 1044 | 3629 | 8729 | 17485 | 22365 | 30416 | 32623 | 43387 |
| 20 | 16 | 0 | 46 | -18 | -91 | -6 | 18 | 760 | 2042 | 5436 | 7664 | 13752 | 19139 | 43345 |
| 20 | 17 | 0 | 119 | 61 | 162 | 870 | 2329 | 7249 | 12388 | 21813 | 26357 | 35562 | 36133 | 45060 |
| 20 | 18 | 0 | 107 | 9 | -46 | 153 | 504 | 2283 | 5219 | 12727 | 17403 | 29693 | 34228 | 45216 |
| 20 | 19 | 0 | 94 | 18 | -6 | 360 | 1034 | 3641 | 7428 | 13990 | 17848 | 28096 | 34042 | 44538 |
| 20 | 20 | 0 | 92 | 15 | -12 | 379 | 980 | 3357 | 6638 | 13307 | 17180 | 27926 | 33868 | 44950 |
| 20 | 21 | 0 | 85 | -3 | -101 | -83 | -89 | 348 | 1105 | 2475 | 3861 | 8143 | 13633 | 44229 |
| 20 | 22 | 0 | 88 | 18 | -95 | -64 | -46 | 464 | 1178 | 3293 | 4797 | 9360 | 14970 | 44415 |
| 20 | 23 | 0 | 89 | 40 | -39 | 116 | 300 | 1865 | 5055 | 12562 | 16710 | 26684 | 32385 | 44187 |
| 20 | 24 | 0 | 55 | -18 | -137 | -137 | -260 | -9 | -40 | -25 | 24 | 684 | 1459 | 43775 |
| 20 | 25 | 0 | 76 | -22 | -101 | -70 | -92 | 311 | 647 | 1553 | 2234 | 5316 | 7523 | 44724 |
| 20 | 26 | 0 | 70 | -21 | -82 | -18 | 113 | 925 | 2488 | 6925 | 9864 | 19319 | 27255 | 45054 |
| 20 | 27 | 0 | 43 | -30 | -146 | -61 | -36 | 550 | 1642 | 3464 | 4997 | 9498 | 14592 | 44868 |
| 20 | 28 | 0 | 52 | -37 | -137 | -186 | -250 | -43 | -101 | -217 | -278 | -281 | -311 | -61 |
| 20 | 29 | 0 | 171 | 229 | 379 | 1331 | 2839 | 7527 | 14543 | 25720 | 29925 | 36038 | 36463 | 44752 |
| 20 | 30 | 0 | 76 | 9 | -74 | -37 | -7 | 564 | 1483 | 3665 | 5576 | 11087 | 16819 | 44626 |
| 20 | 31 | 0 | 55 | 18 | -28 | 296 | 738 | 2569 | 4935 | 8716 | 11124 | 16792 | 22005 | 44296 |
| 20 | 32 | 0 | 64 | 0 | -92 | -9 | -46 | 580 | 1523 | 3705 | 5496 | 10862 | 16117 | 44409 |
| 10 | 1 | 0 | 74 | -55 | -116 | 19 | 254 | 1722 | 3489 | 8876 | 11030 | 20336 | 25399 | 44987 |
| 10 | 2 | 0 | 61 | -55 | -159 | -183 | -183 | 52 | 128 | 409 | 616 | 1852 | 3442 | 44361 |
| 10 | 3 | 0 | 58 | -28 | -159 | -132 | -177 | 119 | 320 | 869 | 1364 | 3531 | 6259 | 44254 |
| 10 | 4 | 0 | 67 | -13 | -95 | -65 | 30 | 814 | 1895 | 4404 | 6384 | 12583 | 19526 | 44260 |
| 10 | 5 | 0 | 61 | -24 | -116 | -146 | -213 | 80 | 296 | 596 | 788 | 1999 | 3123 | 43995 |
| 10 | 6 | 0 | 83 | 9 | -52 | 52 | 269 | 1371 | 3593 | 6846 | 9394 | 16530 | 23925 | 44538 |
| 10 | 7 | 0 | 113 | 98 | 153 | 986 | 1825 | 4792 | 8546 | 12733 | 15312 | 20211 | 24257 | 44041 |
| 10 | 8 | 0 | 55 | -22 | -141 | -107 | -189 | 85 | 244 | 775 | 1218 | 2298 | 3748 | 44220 |
| 10 | 9 | 0 | 131 | 70 | 223 | 1010 | 2268 | 6812 | 13868 | 23528 | 26644 | 35431 | 36352 | 45063 |
| 10 | 10 | 0 | 67 | -52 | -153 | -177 | -189 | -64 | -125 | -223 | -324 | -299 | -336 | -12 |
| 10 | 11 | 0 | 52 | -28 | -165 | -153 | -162 | 201 | 470 | 1340 | 2035 | 4358 | 6342 | 44138 |
| 10 | 12 | 0 | 92 | 0 | -113 | -91 | -119 | 180 | 428 | 1154 | 1764 | 4209 | 7673 | 44547 |
| 10 | 13 | 0 | 110 | 9 | -52 | 18 | 128 | 1022 | 2893 | 8045 | 11082 | 21681 | 28866 | 44700 |
| 10 | 14 | 0 | 55 | -3 | -88 | -88 | -82 | 360 | 995 | 2793 | 4166 | 7530 | 11693 | 44321 |
| 10 | 15 | 0 | 43 | -21 | -134 | -131 | -204 | -27 | -107 | -256 | -281 | -308 | -320 | -6 |
| 10 | 16 | 0 | 80 | -6 | -91 | -24 | -18 | 535 | 1331 | 3428 | 4710 | 9562 | 14867 | 44752 |
| 10 | 17 | 0 | 83 | -42 | -146 | -155 | -228 | 4 | -12 | 0 | 43 | 773 | 1307 | 44389 |
| 10 | 18 | 0 | 70 | -49 | -131 | -113 | -64 | 360 | 852 | 2310 | 3122 | 6699 | 9953 | 44727 |
| 10 | 19 | 0 | 73 | -64 | -168 | -168 | -235 | 9 | 55 | 238 | 442 | 1459 | 3058 | 44126 |
| 10 | 20 | 0 | 92 | -12 | -131 | -152 | -201 | -46 | -49 | -217 | -268 | -296 | -314 | -21 |
| 10 | 21 | 0 | 82 | -12 | -137 | -162 | -204 | -27 | -82 | -204 | -275 | -284 | -293 | -21 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*K. pneumoniae* NCTC 10896 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | 42 | -31 | -122 | -153 | -205 | 79 | 232 | 665 | 1025 | 2740 | 5121 | 44373 |
| 10 | 23 | 0 | 70 | 0 | -119 | -101 | -152 | 98 | 238 | 611 | 1016 | 2430 | 4279 | 43915 |
| 10 | 24 | 0 | 86 | -3 | -115 | -85 | -140 | 208 | 474 | 1252 | 1948 | 4127 | 7203 | 44776 |
| 10 | 25 | 0 | 168 | 110 | 293 | 1075 | 2717 | 5491 | 10063 | 17131 | 20391 | 28143 | 31689 | 44630 |
| 10 | 26 | 0 | 76 | -31 | -95 | -28 | 128 | 1132 | 3049 | 8442 | 11072 | 18629 | 22939 | 44168 |
| 10 | 27 | 0 | 67 | -52 | -161 | -189 | -256 | -45 | -113 | -235 | -305 | -305 | -320 | -58 |
| 10 | 28 | 0 | 92 | 52 | 107 | 559 | 1325 | 4954 | 11430 | 21108 | 26061 | 33874 | 34979 | 44492 |
| 10 | 29 | 0 | 80 | -30 | -100 | -85 | -51 | 413 | 931 | 2378 | 3355 | 6538 | 10136 | 44361 |
| 10 | 30 | 0 | 98 | 61 | 67 | 367 | 913 | 3809 | 8872 | 16701 | 20125 | 29592 | 33502 | 44309 |
| 10 | 31 | 0 | 82 | 27 | -28 | 107 | 281 | 1849 | 4911 | 9339 | 12040 | 18196 | 23345 | 43879 |
| 10 | 32 | 0 | 67 | -7 | -107 | -95 | -174 | 76 | 180 | 488 | 952 | 2115 | 3470 | 44513 |
| 5 | 1 | 0 | 76 | -37 | -138 | -165 | -254 | -43 | -95 | -257 | -296 | -281 | -348 | -89 |
| 5 | 2 | 0 | 92 | -33 | -125 | -149 | -192 | -45 | -79 | -213 | -283 | -280 | -335 | -48 |
| 5 | 3 | 0 | 67 | -46 | -153 | -122 | -110 | 326 | 769 | 2042 | 3043 | 6574 | 10422 | 44300 |
| 5 | 4 | 0 | 95 | -3 | -101 | -107 | -119 | 208 | 507 | 1404 | 2161 | 4484 | 7847 | 44504 |
| 5 | 5 | 0 | 134 | 61 | 73 | 342 | 961 | 4138 | 8991 | 16340 | 20049 | 30035 | 33334 | 44385 |
| 5 | 6 | 0 | 74 | -21 | -112 | -134 | -152 | 223 | 544 | 1301 | 1884 | 4258 | 7844 | 44560 |
| 5 | 7 | 0 | 82 | 18 | -110 | -141 | -193 | -12 | -73 | -214 | -254 | -254 | -315 | -31 |
| 5 | 8 | 0 | 92 | -3 | -103 | -70 | -143 | 190 | 446 | 1133 | 1944 | 4087 | 7417 | 44492 |
| 5 | 9 | 0 | 101 | -27 | -91 | -33 | 34 | 821 | 1761 | 5198 | 7978 | 16615 | 24642 | 44483 |
| 5 | 10 | 0 | 82 | -52 | -150 | -165 | -226 | -49 | -122 | -235 | -318 | -293 | -357 | -80 |
| 5 | 11 | 0 | 49 | -46 | -168 | -190 | -220 | -61 | -113 | -263 | -318 | -321 | -388 | -86 |
| 5 | 12 | 0 | 107 | -3 | -101 | -165 | -229 | -30 | -82 | -223 | -265 | -311 | -323 | -64 |
| 5 | 13 | 0 | 61 | -15 | -140 | -164 | -216 | -58 | -91 | -210 | -296 | -253 | -323 | -58 |
| 5 | 14 | 0 | 92 | 0 | -98 | -153 | -232 | -12 | -85 | -235 | -272 | -259 | -314 | -55 |
| 5 | 15 | 0 | 89 | 15 | -119 | -122 | -220 | -24 | -95 | -232 | -287 | -290 | -345 | -55 |
| 5 | 16 | 0 | 92 | 27 | -101 | -119 | -217 | -6 | -76 | -198 | -262 | -308 | -333 | -55 |
| 5 | 17 | 0 | 92 | -48 | -131 | -152 | -228 | -24 | -100 | -228 | -299 | -286 | -335 | -24 |
| 5 | 18 | 0 | 67 | -27 | -122 | -122 | -134 | 266 | 543 | 1761 | 2057 | 4383 | 6629 | 44541 |
| 5 | 19 | 0 | 61 | -39 | -152 | -189 | -207 | -45 | -116 | -250 | -314 | -329 | -357 | -97 |
| 5 | 20 | 0 | 15 | -70 | -177 | -220 | -262 | -91 | -143 | -265 | -329 | -345 | -384 | -122 |
| 5 | 21 | 0 | 70 | 18 | 9 | 299 | 867 | 3876 | 9195 | 18998 | 22716 | 31567 | 34136 | 44950 |
| 5 | 22 | 0 | 61 | -6 | -128 | -131 | -116 | 274 | 625 | 1776 | 2655 | 6061 | 9876 | 44660 |
| 5 | 23 | 0 | 95 | 6 | -101 | -64 | -131 | 189 | 513 | 1593 | 2228 | 5347 | 7594 | 44221 |
| 5 | 24 | 0 | 92 | -18 | -97 | -103 | -253 | -36 | -94 | -210 | -296 | -308 | -366 | -76 |
| 5 | 25 | 0 | 97 | -49 | -141 | -95 | -147 | 244 | 473 | 1309 | 1767 | 5054 | 7721 | 44486 |
| 5 | 26 | 0 | 82 | -25 | -159 | -189 | -235 | -49 | -110 | -253 | -318 | -278 | -363 | -76 |
| 5 | 27 | 0 | 64 | -24 | -165 | -201 | -262 | -67 | -143 | -287 | -351 | -348 | -390 | -131 |
| 5 | 28 | 0 | 103 | -16 | -119 | -153 | -211 | -43 | -98 | -266 | -299 | -293 | -351 | -101 |
| 5 | 29 | 0 | 100 | -28 | -153 | -159 | -223 | -16 | -86 | -199 | -281 | -269 | -333 | -40 |
| 5 | 30 | 0 | 70 | -13 | -113 | -122 | -138 | 201 | 457 | 1123 | 2072 | 4401 | 7419 | 45004 |
| 5 | 31 | 0 | 67 | 0 | -110 | -134 | -213 | -36 | -88 | -220 | -299 | -302 | -326 | -46 |
| 5 | 32 | 0 | 64 | -43 | -135 | -138 | -235 | -49 | -107 | -241 | -306 | -324 | -382 | -104 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. cloacae* NCTC 11936 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | 149 | -58 | -147 | -128 | -107 | 433 | 2087 | 8683 | 13249 | 23323 | 31255 | 43741 |
| 20 | 2 | 0 | 183 | -34 | -113 | -92 | -52 | 561 | 2695 | 10883 | 16038 | 24620 | 33001 | 44809 |
| 20 | 3 | 0 | 199 | -6 | -82 | -67 | -15 | 580 | 2967 | 12761 | 19051 | 27584 | 34869 | 44358 |
| 20 | 4 | 0 | 198 | 3 | -83 | -16 | 171 | 1245 | 4007 | 14014 | 19593 | 27034 | 34087 | 44449 |
| 20 | 5 | 0 | 244 | 73 | 140 | 741 | 1938 | 5585 | 12568 | 26961 | 30132 | 33434 | 38009 | 43717 |
| 20 | 6 | 0 | 232 | 18 | -73 | -58 | -52 | 235 | 699 | 2548 | 3717 | 6748 | 12486 | 43821 |
| 20 | 7 | 0 | 204 | 40 | -40 | 79 | 345 | 1642 | 5857 | 17836 | 23027 | 29812 | 36407 | 44358 |
| 20 | 8 | 0 | 232 | 27 | -61 | 0 | 174 | 1236 | 3961 | 11896 | 16493 | 24834 | 33257 | 44425 |
| 20 | 9 | 0 | 204 | 6 | -65 | 85 | 955 | 2218 | 6967 | 22133 | 26399 | 32995 | 37930 | 45490 |
| 20 | 10 | 0 | 186 | -9 | -104 | -67 | 3 | 644 | 3266 | 11167 | 12815 | 23430 | 33337 | 45649 |
| 20 | 11 | 0 | 211 | -9 | -100 | -103 | -82 | 156 | 681 | 3168 | 5134 | 10347 | 20913 | 45021 |
| 20 | 12 | 0 | 217 | 16 | -82 | -27 | 22 | 617 | 2842 | 12047 | 15364 | 25027 | 34006 | 45753 |
| 20 | 13 | 0 | 253 | 64 | -16 | 88 | 311 | 1199 | 3427 | 11576 | 16432 | 24995 | 33590 | 45364 |
| 20 | 14 | 0 | 254 | 46 | -39 | -67 | -146 | -24 | -24 | -61 | -195 | -250 | -116 | -9 |
| 20 | 15 | 0 | 238 | 33 | -67 | -83 | -98 | -19 | -31 | -55 | -183 | -257 | -135 | -16 |
| 20 | 16 | 0 | 241 | 89 | 46 | 644 | 2063 | 5292 | 13105 | 26192 | 28869 | 33316 | 38107 | 45432 |
| 20 | 17 | 0 | 201 | -9 | -86 | -55 | 97 | 735 | 3040 | 11753 | 18010 | 27785 | 34646 | 45511 |
| 20 | 18 | 0 | 186 | -3 | -128 | -119 | -198 | -79 | -116 | -122 | -244 | -272 | -177 | -55 |
| 20 | 19 | 0 | 226 | 22 | -100 | -106 | -143 | 37 | 272 | 1127 | 1655 | 3797 | 8534 | 44996 |
| 20 | 20 | 0 | 235 | 49 | -46 | -40 | -76 | 174 | 748 | 4084 | 5994 | 16011 | 25237 | 45511 |
| 20 | 21 | 0 | 274 | 51 | -25 | -65 | -123 | 18 | -16 | -46 | -187 | -245 | -107 | -4 |
| 20 | 22 | 0 | 253 | 64 | -55 | -67 | -144 | -22 | -28 | -46 | -217 | -263 | -159 | -16 |
| 20 | 23 | 0 | 250 | 61 | -46 | -80 | -40 | 125 | 674 | 3317 | 5328 | 11316 | 21040 | 45023 |
| 20 | 24 | 0 | 226 | 27 | -43 | -101 | -162 | -31 | -49 | -70 | -208 | -256 | -131 | -43 |
| 20 | 25 | 0 | 217 | 37 | -67 | -6 | 263 | 1679 | 4926 | 16539 | 22121 | 29483 | 35507 | 45259 |
| 20 | 26 | 0 | 198 | 0 | -92 | -98 | -190 | -74 | -80 | -86 | -226 | -269 | -171 | -55 |
| 20 | 27 | 0 | 241 | 40 | -79 | -100 | -146 | -30 | -67 | -82 | -244 | -284 | -149 | -27 |
| 20 | 28 | 0 | 247 | 55 | -21 | -12 | 52 | 519 | 1621 | 6296 | 9531 | 18849 | 27499 | 45499 |
| 20 | 29 | 0 | 278 | 113 | 3 | 28 | 110 | 833 | 2362 | 7697 | 11494 | 20177 | 28448 | 45222 |
| 20 | 30 | 0 | 247 | 76 | -19 | -61 | -107 | 85 | 296 | 1376 | 2203 | 6632 | 13648 | 44879 |
| 20 | 31 | 0 | 232 | 67 | -46 | -67 | -85 | -3 | -3 | -36 | -204 | -232 | -88 | 34 |
| 20 | 32 | 0 | 232 | 40 | -21 | 143 | 952 | 2011 | 5484 | 12174 | 16179 | 24440 | 31710 | 45096 |
| 10 | 1 | 0 | 198 | 12 | -86 | -68 | -13 | 256 | 744 | 2441 | 3500 | 7532 | 15882 | 44318 |
| 10 | 2 | 0 | 235 | 54 | -34 | -16 | 24 | 473 | 1364 | 4724 | 7477 | 16023 | 25752 | 45447 |
| 10 | 3 | 0 | 229 | 46 | -76 | -82 | -137 | -34 | -52 | -92 | -220 | -305 | -171 | -43 |
| 10 | 4 | 0 | 241 | 55 | -55 | -67 | -128 | -24 | -40 | -58 | -177 | -244 | -134 | -3 |
| 10 | 5 | 0 | 293 | 98 | -3 | -27 | -73 | 24 | 27 | -6 | -146 | -220 | -76 | 67 |
| 10 | 6 | 0 | 251 | 58 | -55 | -55 | -137 | -12 | -30 | -70 | -195 | -250 | -116 | 0 |
| 10 | 7 | 0 | 245 | 65 | -24 | 37 | 232 | 782 | 1737 | 4258 | 5961 | 13387 | 20647 | 44712 |
| 10 | 8 | 0 | 220 | 52 | -61 | -76 | -153 | -31 | -28 | -73 | -223 | -226 | -131 | -28 |
| 10 | 9 | 0 | 202 | 13 | -58 | -12 | 181 | 513 | 1273 | 3626 | 5320 | 11024 | 19228 | 44410 |
| 10 | 10 | 0 | 241 | 36 | -68 | -80 | -159 | -65 | -46 | -74 | -211 | -275 | -138 | -28 |
| 10 | 11 | 0 | 265 | 82 | -31 | -58 | -116 | 3 | -22 | -19 | -186 | -257 | -135 | -3 |
| 10 | 12 | 0 | 289 | 97 | -10 | -19 | -95 | 12 | 30 | -13 | -132 | -135 | 82 | 43988 |
| 10 | 13 | 0 | 281 | 98 | -24 | -27 | -85 | 92 | 208 | 910 | 1612 | 4740 | 11104 | 44679 |
| 10 | 14 | 0 | 269 | 95 | -27 | -27 | -73 | 25 | 15 | -15 | -165 | -238 | -91 | 22 |
| 10 | 15 | 0 | 296 | 92 | 37 | 177 | 540 | 1694 | 3284 | 7758 | 9419 | 15935 | 24581 | 45161 |
| 10 | 16 | 0 | 232 | 48 | -65 | -101 | -159 | -31 | -40 | -52 | -220 | -257 | -129 | -31 |
| 10 | 17 | 0 | 195 | -9 | -113 | -150 | -223 | -89 | -98 | -122 | -244 | -305 | -226 | -107 |
| 10 | 18 | 0 | 228 | 42 | -58 | -86 | -141 | -65 | -52 | -71 | -217 | -275 | -165 | -22 |
| 10 | 19 | 0 | 256 | 101 | -37 | -55 | -76 | 64 | 140 | 440 | 555 | 1557 | 3696 | 44584 |
| 10 | 20 | 0 | 275 | 101 | 0 | -6 | -92 | 116 | 265 | 937 | 1453 | 3705 | 8021 | 45698 |
| 10 | 21 | 0 | 309 | 119 | 16 | 6 | -67 | 104 | 199 | 693 | 986 | 2656 | 5998 | 44907 |

**Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.**

*E. cloacae* NCTC 11936 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | 293 | 113 | -3 | -6 | -76 | 15 | 25 | -18 | -152 | -241 | -94 | 28 |
| 10 | 23 | 0 | 266 | 88 | -6 | 49 | 235 | 851 | 1956 | 4612 | 6067 | 11326 | 17738 | 44727 |
| 10 | 24 | 0 | 248 | 67 | -27 | 9 | 205 | 1075 | 2991 | 8106 | 11134 | 20232 | 27993 | 44550 |
| 10 | 25 | 0 | 155 | -6 | -86 | -125 | -196 | -86 | -83 | -110 | -202 | -263 | -180 | -70 |
| 10 | 26 | 0 | 242 | 46 | -39 | -45 | 3 | 309 | 806 | 2314 | 3596 | 7078 | 13734 | 45240 |
| 10 | 27 | 0 | 290 | 101 | 13 | -12 | -82 | 98 | 180 | 461 | 483 | 1059 | 2109 | 44413 |
| 10 | 28 | 0 | 329 | 134 | 27 | 21 | -95 | 33 | 36 | -13 | -138 | -202 | -80 | 70 |
| 10 | 29 | 0 | 280 | 91 | 3 | -40 | -95 | 70 | 97 | 280 | 271 | 619 | 1535 | 44434 |
| 10 | 30 | 0 | 275 | 58 | -27 | -73 | -88 | 3 | -12 | -33 | -168 | -268 | -149 | -30 |
| 10 | 31 | 0 | 263 | 85 | -24 | -37 | -61 | 6 | -9 | -37 | -168 | -250 | -131 | -15 |
| 10 | 32 | 0 | 251 | 61 | -42 | -73 | -140 | -9 | -24 | -64 | -180 | -253 | -131 | -15 |
| 5 | 1 | 0 | 178 | 19 | -106 | -94 | -103 | 196 | 806 | 4902 | 8372 | 17025 | 25463 | 44908 |
| 5 | 2 | 0 | 220 | 40 | -49 | -82 | -107 | -21 | -36 | -64 | -189 | -247 | -131 | -6 |
| 5 | 3 | 0 | 260 | 61 | -46 | -64 | -152 | -27 | -21 | -58 | -204 | -265 | -149 | -49 |
| 5 | 4 | 0 | 309 | 101 | 0 | 7 | -119 | 37 | 37 | -6 | -164 | -201 | -97 | 3 |
| 5 | 5 | 0 | 277 | 79 | -19 | -37 | -101 | 30 | 15 | -31 | -168 | -248 | -122 | -34 |
| 5 | 6 | 0 | 241 | 61 | -46 | -68 | -110 | 0 | -13 | -64 | -199 | -287 | -159 | -49 |
| 5 | 7 | 0 | 248 | 40 | -42 | -64 | -76 | -12 | -30 | -55 | -195 | -256 | -158 | -24 |
| 5 | 8 | 0 | 269 | 86 | -30 | -49 | -134 | 6 | -21 | -46 | -186 | -281 | -119 | -42 |
| 5 | 9 | 0 | 177 | -3 | -110 | -141 | -193 | -64 | -64 | -61 | -138 | 3 | 552 | 44049 |
| 5 | 10 | 0 | 216 | 15 | -92 | -101 | -126 | 64 | 387 | 1828 | 3570 | 10437 | 18818 | 45212 |
| 5 | 11 | 0 | 226 | 46 | -61 | -85 | -143 | -52 | -43 | -70 | -235 | -293 | -162 | -61 |
| 5 | 12 | 0 | 259 | 82 | -52 | -34 | -55 | 345 | 980 | 2814 | 4038 | 8909 | 16722 | 45044 |
| 5 | 13 | 0 | 245 | 68 | -27 | -58 | -106 | 7 | -6 | -21 | -195 | -259 | -125 | -21 |
| 5 | 14 | 0 | 232 | 58 | -71 | -77 | -132 | -19 | -25 | -55 | -180 | -275 | -153 | -55 |
| 5 | 15 | 0 | 257 | 80 | -12 | 107 | 406 | 1777 | 4768 | 11619 | 14345 | 22665 | 31103 | 44828 |
| 5 | 16 | 0 | 281 | 74 | -48 | -61 | -125 | 0 | -15 | -54 | -189 | -256 | -143 | -15 |
| 5 | 17 | 0 | 184 | 10 | -109 | -106 | -128 | 28 | 232 | 1893 | 2805 | 9816 | 17601 | 44715 |
| 5 | 18 | 0 | 192 | 12 | -104 | -138 | -168 | -77 | -89 | -113 | -266 | -312 | -186 | -83 |
| 5 | 19 | 0 | 211 | 34 | -89 | -104 | -168 | -52 | -58 | -92 | -241 | -290 | -180 | -95 |
| 5 | 20 | 0 | 235 | 46 | -67 | -88 | -146 | -3 | -43 | -52 | -208 | -259 | -165 | -27 |
| 5 | 21 | 0 | 269 | 73 | -39 | -64 | -113 | 22 | -3 | -39 | -192 | -278 | -131 | -21 |
| 5 | 22 | 0 | 238 | 58 | -37 | -58 | -113 | -21 | -43 | -61 | -162 | -265 | -140 | -30 |
| 5 | 23 | 0 | 245 | 61 | -12 | 16 | 159 | 834 | 2790 | 9623 | 12636 | 21450 | 30377 | 44596 |
| 5 | 24 | 0 | 229 | 64 | -51 | -55 | -100 | 0 | -33 | -45 | -183 | -268 | -149 | -18 |
| 5 | 25 | 0 | 183 | 0 | -98 | -107 | -159 | -76 | -95 | -122 | -272 | -311 | -177 | -79 |
| 5 | 26 | 0 | 205 | 3 | -97 | -134 | -146 | -79 | -91 | -103 | -241 | -308 | -207 | -94 |
| 5 | 27 | 0 | 220 | 25 | -88 | -103 | -161 | -58 | -70 | -125 | -235 | -290 | -186 | -79 |
| 5 | 28 | 0 | 217 | 49 | -55 | -98 | -143 | -18 | -49 | -79 | -220 | -287 | -180 | -52 |
| 5 | 29 | 0 | 275 | 95 | 9 | 58 | 232 | 705 | 1624 | 4179 | 5170 | 9016 | 14949 | 44639 |
| 5 | 30 | 0 | 256 | 67 | -43 | -68 | -110 | -10 | -28 | -55 | -193 | -266 | -141 | -37 |
| 5 | 31 | 0 | 214 | 33 | -67 | -107 | -122 | -55 | -73 | -95 | -250 | -308 | -177 | -70 |
| 5 | 32 | 0 | 235 | 42 | -61 | -83 | -98 | -31 | -58 | -80 | -214 | -296 | -168 | -65 |

## Appendix 5.4: Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. coli* NCIMB 10213 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -40 | -351 | -317 | -333 | -330 | -360 | -418 | -333 | -378 | -452 | -528 | -464 |
| 20 | 2 | 0 | -49 | -351 | -296 | -308 | -308 | -314 | -427 | -293 | -375 | -415 | -479 | -381 |
| 20 | 3 | 0 | -28 | -345 | -281 | -293 | -290 | -324 | -400 | -284 | -363 | -403 | -483 | -385 |
| 20 | 4 | 0 | -49 | -345 | -302 | -305 | -305 | -320 | -430 | -296 | -387 | -406 | -485 | -418 |
| 20 | 5 | 0 | -73 | -342 | -317 | -302 | -311 | -335 | -415 | -311 | -375 | -427 | -491 | -390 |
| 20 | 6 | 0 | -85 | -332 | -308 | -302 | -320 | -348 | -421 | -302 | -384 | -442 | -500 | -427 |
| 20 | 7 | 0 | -67 | -327 | -311 | -287 | -327 | -333 | -406 | -290 | -369 | -418 | -461 | -400 |
| 20 | 8 | 0 | -64 | -326 | -287 | -290 | -308 | -320 | -394 | -284 | -357 | -406 | -473 | -391 |
| 20 | 9 | 0 | -27 | -311 | -268 | -241 | -268 | -265 | -345 | -217 | -296 | -333 | -387 | -299 |
| 20 | 10 | 0 | -3 | -281 | -232 | -235 | -214 | -232 | -333 | -198 | -263 | -299 | -366 | -214 |
| 20 | 11 | 0 | -3 | -287 | -216 | -222 | -226 | -226 | -280 | -119 | -103 | 46 | 681 | 38864 |
| 20 | 12 | 0 | -31 | -324 | -284 | -269 | -266 | -266 | -357 | -235 | -299 | -348 | -403 | -306 |
| 20 | 13 | 0 | -6 | -317 | -265 | -262 | -271 | -268 | -351 | -232 | -274 | -357 | -384 | -280 |
| 20 | 14 | 0 | -19 | -290 | -232 | -229 | -250 | -260 | -342 | -232 | -275 | -330 | -388 | -302 |
| 20 | 15 | 0 | -64 | -308 | -290 | -244 | -281 | -287 | -366 | -226 | -296 | -354 | -372 | -302 |
| 20 | 16 | 0 | -24 | -308 | -262 | -238 | -253 | -281 | -351 | -195 | -272 | -320 | -369 | -262 |
| 20 | 17 | 0 | -15 | -290 | -247 | -226 | -256 | -220 | -317 | -189 | -256 | -290 | -369 | -247 |
| 20 | 18 | 0 | 15 | -284 | -232 | -174 | -223 | -220 | -321 | -156 | -226 | -266 | -318 | -211 |
| 20 | 19 | 0 | 31 | -253 | -213 | -189 | -198 | -198 | -274 | -146 | -180 | -238 | -323 | -216 |
| 20 | 20 | 0 | -15 | -308 | -259 | -262 | -256 | -247 | -338 | -207 | -259 | -299 | -360 | -289 |
| 20 | 21 | 0 | -15 | -278 | -217 | -217 | -201 | -205 | -284 | -150 | -214 | -263 | -299 | -253 |
| 20 | 22 | 0 | -55 | -293 | -262 | -244 | -250 | -262 | -326 | -192 | -244 | -302 | -363 | -290 |
| 20 | 23 | 0 | -40 | -284 | -247 | -232 | -244 | -256 | -311 | -186 | -250 | -287 | -330 | -247 |
| 20 | 24 | 0 | -45 | -311 | -259 | -244 | -244 | -274 | -360 | -198 | -250 | -305 | -357 | -238 |
| 20 | 25 | 0 | -15 | -308 | -244 | -213 | -256 | -253 | -326 | -177 | -241 | -274 | -357 | -265 |
| 20 | 26 | 0 | 3 | -284 | -217 | -232 | -223 | -220 | -296 | -171 | -207 | -259 | -305 | -226 |
| 20 | 27 | 0 | 3 | -275 | -214 | -199 | -211 | -214 | -281 | -153 | -189 | -244 | -305 | -214 |
| 20 | 28 | 0 | 18 | -278 | -208 | -208 | -205 | -208 | -269 | -153 | -195 | -263 | -290 | -211 |
| 20 | 29 | 0 | 6 | -257 | -208 | -196 | -187 | -211 | -269 | -138 | -190 | -260 | -293 | -217 |
| 20 | 30 | 0 | 18 | -260 | -250 | -214 | -232 | -211 | -308 | -168 | -205 | -254 | -296 | -235 |
| 20 | 31 | 0 | -12 | -250 | -210 | -189 | -213 | -216 | -283 | -161 | -183 | -219 | -250 | -204 |
| 20 | 32 | 0 | -24 | -284 | -244 | -216 | -244 | -244 | -311 | -168 | -235 | -265 | -317 | -226 |
| 10 | 1 | 0 | -12 | -284 | -253 | -220 | -244 | -238 | -281 | -204 | -244 | -268 | -323 | -256 |
| 10 | 2 | 0 | -9 | -308 | -235 | -216 | -220 | -223 | -314 | -140 | -216 | -284 | -320 | -207 |
| 10 | 3 | 0 | 21 | -253 | -189 | -171 | -186 | -156 | -259 | -116 | -165 | -214 | -256 | -192 |
| 10 | 4 | 0 | 0 | -287 | -229 | -217 | -211 | -159 | 86 | 1630 | 3949 | 6986 | 13682 | 41541 |
| 10 | 5 | 0 | -9 | -269 | -226 | -196 | -208 | -205 | -272 | -138 | -196 | -251 | -290 | -202 |
| 10 | 6 | 0 | -7 | -278 | -217 | -202 | -226 | -229 | -278 | -156 | -199 | -239 | -290 | -223 |
| 10 | 7 | 0 | -22 | -248 | -214 | -196 | -232 | -217 | -278 | -150 | -199 | -245 | -242 | -211 |
| 10 | 8 | 0 | -39 | -308 | -250 | -222 | -247 | -268 | -323 | -186 | -228 | -274 | -311 | -241 |
| 10 | 9 | 0 | -15 | -269 | -223 | -192 | -226 | -220 | -302 | -171 | -220 | -250 | -318 | -223 |
| 10 | 10 | 0 | 40 | -244 | -220 | -189 | -211 | -202 | -287 | -128 | -189 | -238 | -293 | -168 |
| 10 | 11 | 0 | 12 | -263 | -205 | -190 | -205 | -180 | -281 | -147 | -174 | -238 | -278 | -235 |
| 10 | 12 | 0 | -16 | -275 | -217 | -208 | -211 | -211 | -309 | -135 | -193 | -266 | -281 | -190 |
| 10 | 13 | 0 | -31 | -284 | -241 | -223 | -220 | -199 | -284 | -165 | -199 | -254 | -305 | -238 |
| 10 | 14 | 0 | 7 | -256 | -204 | -192 | -201 | -213 | -293 | -128 | -174 | -204 | -268 | -222 |
| 10 | 15 | 0 | -43 | -281 | -226 | -217 | -208 | -232 | -287 | -156 | -199 | -238 | -263 | -202 |
| 10 | 16 | 0 | -64 | -302 | -256 | -232 | -235 | -238 | -299 | -183 | -223 | -265 | -314 | -235 |
| 10 | 17 | 0 | 0 | -266 | -202 | -214 | -232 | -214 | -287 | -153 | -214 | -232 | -324 | -211 |
| 10 | 18 | 0 | 3 | -287 | -226 | -201 | -208 | -214 | -284 | -140 | -211 | -266 | -305 | -189 |
| 10 | 19 | 0 | -3 | -281 | -223 | -211 | -205 | -223 | -260 | -156 | -214 | -248 | -272 | -245 |
| 10 | 20 | 0 | -9 | -254 | -220 | -199 | -208 | -199 | -284 | -147 | -211 | -251 | -293 | -193 |
| 10 | 21 | 0 | -30 | -296 | -247 | -238 | -211 | -235 | -293 | -156 | -204 | -241 | -305 | -226 |

**Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.**

*E. coli* NCIMB 10213 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -19 | -269 | -229 | -202 | -199 | -211 | -284 | -150 | -183 | -202 | -296 | -214 |
| 10 | 23 | 0 | -22 | -275 | -223 | -217 | -223 | -217 | -299 | -168 | -205 | -247 | -260 | -199 |
| 10 | 24 | 0 | -40 | -299 | -229 | -186 | -220 | -250 | -293 | -177 | -211 | -253 | -299 | -198 |
| 10 | 25 | 0 | -21 | -272 | -241 | -226 | -214 | -229 | -317 | -174 | -211 | -223 | -320 | -229 |
| 10 | 26 | 0 | -3 | -287 | -226 | -223 | -223 | -226 | -299 | -159 | -204 | -253 | -314 | -183 |
| 10 | 27 | 0 | -9 | -262 | -222 | -201 | -210 | -219 | -277 | -158 | -192 | -238 | -293 | -216 |
| 10 | 28 | 0 | 25 | -235 | -204 | -177 | -177 | -195 | -271 | -100 | -186 | -235 | -280 | -192 |
| 10 | 29 | 0 | -34 | -299 | -253 | -232 | -226 | -232 | -311 | -186 | -226 | -266 | -317 | -247 |
| 10 | 30 | 0 | -24 | -296 | -238 | -204 | -210 | -213 | -280 | -146 | -189 | -225 | -293 | -219 |
| 10 | 31 | 0 | -33 | -274 | -225 | -201 | -216 | -198 | -283 | -152 | -183 | -238 | -238 | -195 |
| 10 | 32 | 0 | -46 | -290 | -220 | -220 | -223 | -247 | -287 | -159 | -223 | -256 | -284 | -207 |
| 5 | 1 | 0 | -15 | -278 | -250 | -223 | -232 | -211 | -305 | -180 | -223 | -232 | -321 | -226 |
| 5 | 2 | 0 | -6 | -308 | -238 | -217 | -226 | -205 | -299 | -156 | -205 | -241 | -293 | -189 |
| 5 | 3 | 0 | -30 | -314 | -232 | -232 | -244 | -250 | -326 | -162 | -229 | -287 | -314 | -241 |
| 5 | 4 | 0 | -9 | -287 | -229 | -235 | -214 | -232 | -281 | -144 | -226 | -244 | -318 | -226 |
| 5 | 5 | 0 | -13 | -278 | -211 | -205 | -211 | -205 | -284 | -159 | -211 | -220 | -290 | -208 |
| 5 | 6 | 0 | -25 | -296 | -232 | -214 | -214 | -235 | -287 | -159 | -214 | -254 | -309 | -208 |
| 5 | 7 | 0 | -46 | -299 | -259 | -232 | -247 | -259 | -302 | -159 | -204 | -247 | -253 | -195 |
| 5 | 8 | 0 | -46 | -296 | -250 | -232 | -235 | -265 | -314 | -180 | -220 | -247 | -302 | -226 |
| 5 | 9 | 0 | -7 | -278 | -232 | -208 | -217 | -193 | -284 | -159 | -217 | -217 | -306 | -196 |
| 5 | 10 | 0 | -6 | -293 | -241 | -223 | -214 | -217 | -296 | -159 | -204 | -241 | -299 | -192 |
| 5 | 11 | 0 | -21 | -305 | -259 | -205 | -223 | -238 | -299 | -174 | -217 | -275 | -281 | -229 |
| 5 | 12 | 0 | -15 | -266 | -223 | -202 | -208 | -196 | -278 | -138 | -196 | -235 | -312 | -208 |
| 5 | 13 | 0 | -36 | -299 | -259 | -241 | -232 | -271 | -299 | -186 | -241 | -274 | -314 | -232 |
| 5 | 14 | 0 | -9 | -250 | -216 | -213 | -201 | -219 | -259 | -134 | -155 | -235 | -274 | -192 |
| 5 | 15 | 0 | -25 | -272 | -238 | -195 | -223 | -238 | -299 | -150 | -205 | -256 | -281 | -214 |
| 5 | 16 | 0 | -43 | -308 | -250 | -244 | -250 | -241 | -308 | -171 | -223 | -281 | -308 | -223 |
| 5 | 17 | 0 | 15 | -260 | -217 | -205 | -202 | -177 | -269 | -119 | -199 | -217 | -312 | -177 |
| 5 | 18 | 0 | -21 | -293 | -232 | -220 | -213 | -223 | -311 | -155 | -201 | -250 | -296 | -174 |
| 5 | 19 | 0 | -12 | -296 | -244 | -192 | -219 | -219 | -256 | -137 | -192 | -235 | -277 | -189 |
| 5 | 20 | 0 | -21 | -290 | -244 | -229 | -205 | -229 | -290 | -147 | -201 | -247 | -296 | -201 |
| 5 | 21 | 0 | -55 | -281 | -259 | -226 | -238 | -229 | -317 | -174 | -232 | -262 | -324 | -229 |
| 5 | 22 | 0 | -64 | -293 | -254 | -251 | -245 | -235 | -312 | -177 | -217 | -278 | -309 | -217 |
| 5 | 23 | 0 | -16 | -275 | -232 | -196 | -208 | -244 | -290 | -147 | -208 | -260 | -287 | -193 |
| 5 | 24 | 0 | -42 | -314 | -265 | -247 | -235 | -265 | -314 | -177 | -235 | -265 | -296 | -204 |
| 5 | 25 | 0 | -15 | -293 | -222 | -219 | -222 | -213 | -274 | -146 | -225 | -228 | -317 | -195 |
| 5 | 26 | 0 | -12 | -326 | -265 | -253 | -244 | -229 | -326 | -168 | -238 | -259 | -335 | -201 |
| 5 | 27 | 0 | -9 | -308 | -247 | -259 | -238 | -250 | -323 | -177 | -235 | -268 | -317 | -189 |
| 5 | 28 | 0 | -51 | -290 | -244 | -247 | -241 | -232 | -293 | -137 | -207 | -247 | -299 | -189 |
| 5 | 29 | 0 | -27 | -293 | -253 | -244 | -220 | -226 | -302 | -165 | -226 | -265 | -348 | -195 |
| 5 | 30 | 0 | -52 | -306 | -263 | -257 | -263 | -251 | -324 | -190 | -235 | -287 | -333 | -223 |
| 5 | 31 | 0 | -30 | -302 | -262 | -247 | -241 | -256 | -305 | -180 | -235 | -284 | -323 | -213 |
| 5 | 32 | 0 | -39 | -326 | -253 | -235 | -256 | -268 | -317 | -189 | -235 | -256 | -299 | -213 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. aerogenes* NCIMB 10102 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | 92 | 105.5 | 119 | 55 | 153 | 202 | 583 | 1526 | 6892 | 12327 | 17845 | 48844 |
| 20 | 2 | 0 | 98 | 101 | 104 | 101 | 171 | 269 | 962 | 3135 | 9935 | 15208 | 21456 | 48997 |
| 20 | 3 | 0 | 101 | 101 | 101 | 86 | 205 | 712 | 2573 | 5805 | 13448 | 20110 | 26925 | 48176 |
| 20 | 4 | 0 | 70 | 72 | 74 | 74 | 159 | 412 | 1410 | 2625 | 6480 | 12193 | 19603 | 47474 |
| 20 | 5 | 0 | 97 | 88 | 79 | 158 | 305 | 805 | 2029 | 3866 | 11313 | 19459 | 26280 | 46652 |
| 20 | 6 | 0 | 98 | 72 | 46 | 92 | 104 | 28 | 159 | 351 | 2005 | 6162 | 14656 | 45515 |
| 20 | 7 | 0 | 97 | 71 | 45 | 42 | 103 | 61 | 222 | 589 | 2591 | 6699 | 14106 | 42728 |
| 20 | 8 | 0 | 34 | 9.5 | -15 | -30 | 21 | -12 | 290 | 946 | 3281 | 7905 | 17485 | 38898 |
| 20 | 9 | 0 | 57 | 95.5 | 134 | 82 | 213 | 396 | 1382 | 3341 | 9769 | 14640 | 20448 | 49173 |
| 20 | 10 | 0 | 54 | 80 | 106 | 79 | 192 | 418 | 1538 | 3735 | 10038 | 15269 | 20988 | 48880 |
| 20 | 11 | 0 | 107 | 113 | 119 | 134 | 201 | 430 | 1349 | 3473 | 10191 | 15657 | 21663 | 48402 |
| 20 | 12 | 0 | 61 | 104 | 147 | 131 | 217 | 272 | 998 | 2802 | 7511 | 11854 | 17864 | 48603 |
| 20 | 13 | 0 | 143 | 134 | 125 | 125 | 180 | 348 | 1175 | 2826 | 7733 | 13673 | 20848 | 47846 |
| 20 | 14 | 0 | 156 | 153 | 150 | 168 | 266 | 348 | 1017 | 2527 | 7246 | 14137 | 23354 | 49602 |
| 20 | 15 | 0 | 159 | 154 | 149 | 149 | 220 | 223 | 485 | 897 | 2963 | 7331 | 14732 | 45948 |
| 20 | 16 | 0 | 64 | 49 | 34 | 25 | 80 | -52 | 43 | 83 | 598 | 1838 | 4935 | 42826 |
| 20 | 17 | 0 | 71 | 86 | 101 | 104 | 184 | 324 | 867 | 2155 | 7347 | 11751 | 16603 | 49214 |
| 20 | 18 | 0 | 57 | 84.5 | 112 | 100 | 149 | 253 | 705 | 1681 | 6027 | 10215 | 15171 | 49124 |
| 20 | 19 | 0 | 86 | 92 | 98 | 95 | 186 | 330 | 1114 | 2915 | 9040 | 13493 | 18730 | 48350 |
| 20 | 20 | 0 | 43 | 79.5 | 116 | 110 | 202 | 199 | 467 | 1111 | 4023 | 7899 | 12614 | 48841 |
| 20 | 21 | 0 | 152 | 146 | 140 | 128 | 204 | 283 | 1010 | 2756 | 7401 | 12235 | 17738 | 48026 |
| 20 | 22 | 0 | 158 | 135 | 112 | 155 | 268 | 399 | 1391 | 3091 | 6644 | 12061 | 18693 | 47827 |
| 20 | 23 | 0 | 171 | 151 | 131 | 134 | 257 | 308 | 705 | 1264 | 3541 | 8173 | 15385 | 46928 |
| 20 | 24 | 0 | 125 | 105 | 85 | 73 | 152 | 146 | 509 | 1358 | 5396 | 12498 | 22090 | 44858 |
| 20 | 25 | 0 | 64 | 93 | 122 | 95 | 126 | 245 | 647 | 1560 | 6053 | 10875 | 15581 | 49226 |
| 20 | 26 | 0 | 83 | 93.5 | 104 | 76 | 150 | 250 | 629 | 1715 | 6229 | 11021 | 16356 | 49061 |
| 20 | 27 | 0 | 67 | 79.5 | 92 | 119 | 171 | 275 | 855 | 2265 | 8433 | 12779 | 17931 | 48610 |
| 20 | 28 | 0 | 34 | 76.5 | 119 | 101 | 192 | 287 | 794 | 2265 | 7233 | 11683 | 16951 | 48362 |
| 20 | 29 | 0 | 147 | 147 | 147 | 156 | 177 | 150 | 312 | 745 | 3571 | 6989 | 11558 | 47694 |
| 20 | 30 | 0 | 159 | 148 | 137 | 177 | 244 | 208 | 574 | 1633 | 5054 | 9428 | 15361 | 47569 |
| 20 | 31 | 0 | 159 | 144 | 129 | 132 | 235 | 257 | 620 | 1041 | 2625 | 5714 | 11989 | 46821 |
| 20 | 32 | 0 | 119 | 99 | 79 | 76 | 140 | 143 | 522 | 1422 | 5847 | 12598 | 23415 | 45386 |
| 10 | 1 | 0 | 104 | 102.5 | 101 | 104 | 104 | 183 | 391 | 757 | 3312 | 7029 | 10960 | 49305 |
| 10 | 2 | 0 | 82 | 96 | 110 | 91 | 204 | 296 | 766 | 2011 | 7267 | 11680 | 16728 | 48701 |
| 10 | 3 | 0 | 98 | 110 | 122 | 89 | 144 | 104 | 177 | 293 | 1288 | 3348 | 6254 | 47892 |
| 10 | 4 | 0 | 82 | 109.5 | 137 | 88 | 134 | 106 | 100 | 39 | 27 | 70 | 64 | -263 |
| 10 | 5 | 0 | 131 | 129.5 | 128 | 140 | 156 | 134 | 278 | 537 | 3241 | 6220 | 10581 | 47562 |
| 10 | 6 | 0 | 147 | 125.5 | 104 | 141 | 223 | 126 | 364 | 788 | 3504 | 7249 | 11769 | 47599 |
| 10 | 7 | 0 | 152 | 128 | 104 | 122 | 195 | 192 | 354 | 503 | 1157 | 1834 | 2887 | 46256 |
| 10 | 8 | 0 | 125 | 96 | 67 | 58 | 125 | 12 | 55 | 58 | 332 | 1266 | 3653 | 44193 |
| 10 | 9 | 0 | 83 | 95 | 107 | 113 | 125 | 272 | 687 | 1828 | 6223 | 10615 | 15507 | 49263 |
| 10 | 10 | 0 | 74 | 95 | 116 | 104 | 180 | 336 | 867 | 2073 | 6828 | 10984 | 15904 | 49183 |
| 10 | 11 | 0 | 64 | 86.5 | 109 | 134 | 207 | 250 | 598 | 1419 | 5560 | 9513 | 14240 | 48123 |
| 10 | 12 | 0 | 97 | 123 | 149 | 91 | 159 | 290 | 748 | 1913 | 6794 | 11637 | 17173 | 48154 |
| 10 | 13 | 0 | 135 | 139.5 | 144 | 132 | 144 | 122 | 226 | 412 | 2393 | 5482 | 9657 | 47868 |
| 10 | 14 | 0 | 137 | 124.5 | 112 | 152 | 216 | 216 | 717 | 2047 | 5808 | 9867 | 14851 | 47495 |
| 10 | 15 | 0 | 170 | 152 | 134 | 131 | 253 | 225 | 467 | 683 | 1590 | 3021 | 6070 | 46475 |
| 10 | 16 | 0 | 104 | 75 | 46 | 27 | 104 | 40 | 159 | 281 | 1511 | 4114 | 9952 | 44278 |
| 10 | 17 | 0 | 100 | 109.5 | 119 | 143 | 216 | 406 | 1159 | 2844 | 8313 | 12849 | 17439 | 48682 |
| 10 | 18 | 0 | 92 | 104 | 116 | 113 | 95 | 125 | 281 | 495 | 2124 | 4584 | 8213 | 48970 |
| 10 | 19 | 0 | 70 | 89.5 | 109 | 143 | 152 | 88 | 140 | 235 | 900 | 2597 | 5389 | 48148 |
| 10 | 20 | 0 | 101 | 124 | 147 | 77 | 98 | 116 | 159 | 214 | 779 | 2344 | 5192 | 47773 |
| 10 | 21 | 0 | 128 | 129.5 | 131 | 128 | 171 | 171 | 348 | 772 | 3858 | 7856 | 12828 | 47526 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. aerogenes* NCIMB 10102 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | 125 | 117.5 | 110 | 153 | 196 | 74 | 159 | 205 | 1072 | 2888 | 5668 | 47413 |
| 10 | 23 | 0 | 192 | 164.5 | 137 | 143 | 244 | 308 | 668 | 1294 | 3424 | 7010 | 13959 | 46744 |
| 10 | 24 | 0 | 97 | 76 | 55 | 52 | 103 | 6 | 152 | 268 | 1446 | 4260 | 10935 | 44980 |
| 10 | 25 | 0 | 79 | 82.5 | 86 | 70 | 92 | 76 | 177 | 299 | 1059 | 2826 | 5369 | 48655 |
| 10 | 26 | 0 | 82 | 90 | 98 | 107 | 119 | 207 | 558 | 1300 | 4682 | 8503 | 13407 | 48588 |
| 10 | 27 | 0 | 80 | 80 | 80 | 150 | 95 | 101 | 287 | 589 | 2637 | 5677 | 9828 | 47810 |
| 10 | 28 | 0 | 119 | 117.5 | 116 | 94 | 106 | 219 | 454 | 1056 | 3934 | 7798 | 12452 | 47944 |
| 10 | 29 | 0 | 106 | 97 | 88 | 116 | 143 | 177 | 464 | 1114 | 4413 | 7807 | 13215 | 47916 |
| 10 | 30 | 0 | 143 | 131 | 119 | 171 | 214 | 201 | 595 | 1483 | 4697 | 8460 | 13136 | 47248 |
| 10 | 31 | 0 | 143 | 140 | 137 | 116 | 195 | 220 | 525 | 1111 | 2896 | 6183 | 12974 | 46436 |
| 10 | 32 | 0 | 107 | 76.5 | 46 | 62 | 95 | 25 | 89 | 92 | 629 | 2109 | 6062 | 44660 |
| 5 | 1 | 0 | 83 | 80 | 77 | 70 | 55 | 74 | 156 | 269 | 943 | 2701 | 5457 | 48625 |
| 5 | 2 | 0 | 80 | 81.5 | 83 | 126 | 80 | 86 | 208 | 373 | 1835 | 4230 | 8039 | 48951 |
| 5 | 3 | 0 | 100 | 100 | 100 | 177 | 116 | 189 | 451 | 970 | 3809 | 6766 | 11521 | 48423 |
| 5 | 4 | 0 | 107 | 126.5 | 146 | 88 | 103 | 140 | 226 | 387 | 1575 | 3967 | 7514 | 48160 |
| 5 | 5 | 0 | 113 | 117.5 | 122 | 119 | 153 | 128 | 205 | 284 | 1191 | 3422 | 7429 | 47581 |
| 5 | 6 | 0 | 146 | 117 | 88 | 131 | 161 | 64 | 64 | 15 | 49 | 79 | 58 | -284 |
| 5 | 7 | 0 | 183 | 158.5 | 134 | 107 | 207 | 189 | 406 | 744 | 2002 | 4089 | 9501 | 46589 |
| 5 | 8 | 0 | 126 | 95.5 | 65 | 43 | 95 | 4 | 22 | -48 | -64 | 10 | -51 | -415 |
| 5 | 9 | 0 | 116 | 100.5 | 85 | 113 | 79 | 52 | 61 | 46 | 43 | 85 | 33 | -266 |
| 5 | 10 | 0 | 55 | 61 | 67 | 149 | 91 | 198 | 500 | 1089 | 3882 | 7129 | 12025 | 49067 |
| 5 | 11 | 0 | 122 | 106.5 | 91 | 171 | 116 | 186 | 381 | 836 | 3351 | 6562 | 11494 | 48633 |
| 5 | 12 | 0 | 98 | 120.5 | 143 | 76 | 110 | 125 | 192 | 357 | 1554 | 3824 | 7691 | 48237 |
| 5 | 13 | 0 | 125 | 129.5 | 134 | 156 | 195 | 265 | 641 | 1596 | 5240 | 9580 | 14537 | 48182 |
| 5 | 14 | 0 | 146 | 118.5 | 91 | 171 | 210 | 155 | 344 | 628 | 2344 | 4742 | 8493 | 47699 |
| 5 | 15 | 0 | 140 | 128 | 116 | 128 | 186 | 98 | 162 | 204 | 577 | 1175 | 2600 | 46616 |
| 5 | 16 | 0 | 85 | 60.5 | 36 | 21 | 67 | -3 | 33 | 64 | 534 | 1721 | 4806 | 43686 |
| 5 | 17 | 0 | 55 | 55 | 55 | 80 | 58 | 40 | 98 | 129 | 473 | 1569 | 4175 | 49223 |
| 5 | 18 | 0 | 42 | 46.5 | 51 | 158 | 109 | 189 | 436 | 1098 | 3882 | 6970 | 10947 | 49164 |
| 5 | 19 | 0 | 76 | 70 | 64 | 119 | 137 | 134 | 344 | 927 | 3528 | 6561 | 10962 | 48639 |
| 5 | 20 | 0 | 31 | 88.5 | 146 | 61 | 88 | 101 | 128 | 162 | 598 | 1569 | 4038 | 48850 |
| 5 | 21 | 0 | 80 | 101.5 | 123 | 101 | 107 | 77 | 101 | 10 | 49 | 144 | 199 | 47877 |
| 5 | 22 | 0 | 100 | 91 | 82 | 122 | 192 | 165 | 442 | 1016 | 3577 | 6784 | 11744 | 48386 |
| 5 | 23 | 0 | 131 | 111.5 | 92 | 76 | 156 | 52 | 95 | 6 | 223 | 501 | 1300 | 47346 |
| 5 | 24 | 0 | 110 | 91.5 | 73 | 24 | 100 | -22 | 15 | -34 | -61 | -9 | -43 | -452 |
| 5 | 25 | 0 | 77 | 70.5 | 64 | 92 | 113 | 220 | 608 | 1334 | 4618 | 8259 | 13158 | 48729 |
| 5 | 26 | 0 | 77 | 73.5 | 70 | 156 | 135 | 186 | 385 | 864 | 3452 | 6678 | 11045 | 48964 |
| 5 | 27 | 0 | 143 | 128 | 113 | 140 | 171 | 125 | 283 | 515 | 2218 | 4831 | 9351 | 48664 |
| 5 | 28 | 0 | 36 | 86.5 | 137 | 61 | 79 | 112 | 122 | 210 | 988 | 2591 | 5930 | 48560 |
| 5 | 29 | 0 | 67 | 88 | 109 | 158 | 143 | 238 | 586 | 1666 | 5646 | 9745 | 15113 | 48108 |
| 5 | 30 | 0 | 146 | 121.5 | 97 | 134 | 180 | 88 | 110 | 15 | 67 | 73 | 27 | -296 |
| 5 | 31 | 0 | 162 | 143.5 | 125 | 131 | 208 | 107 | 214 | 351 | 992 | 1953 | 4349 | 46598 |
| 5 | 32 | 0 | 125 | 106.5 | 88 | 39 | 97 | 6 | 27 | -13 | -43 | 30 | -19 | -425 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*C. freundii* FRHCFR2 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -528 | -253 | -308 | -272 | -15 | 641 | 2194 | 5649 | 12251 | 21977 | 39682 | 54158 |
| 20 | 2 | 0 | -553 | -260 | -263 | -220 | 219 | 1025 | 2753 | 6912 | 15074 | 26931 | 43250 | 53675 |
| 20 | 3 | 0 | -567 | -268 | -287 | -329 | -290 | -192 | 80 | 1767 | 7227 | 17067 | 37055 | 53065 |
| 20 | 4 | 0 | -549 | -244 | -278 | -342 | -314 | -339 | -357 | -339 | -369 | -397 | -409 | -366 |
| 20 | 5 | 0 | -534 | -199 | -226 | -269 | -159 | 174 | 1181 | 4013 | 10633 | 21046 | 35257 | 55171 |
| 20 | 6 | 0 | -562 | -263 | -266 | -360 | -327 | -327 | -296 | -125 | 409 | 1886 | 8054 | 50297 |
| 20 | 7 | 0 | -635 | -284 | -324 | -409 | -360 | -382 | -418 | -324 | -491 | -446 | -528 | -403 |
| 20 | 8 | 0 | -635 | -296 | -354 | -425 | -394 | -415 | -418 | -208 | -12 | 815 | 3827 | 44641 |
| 20 | 9 | 0 | -494 | -220 | -253 | -256 | -192 | 119 | 1395 | 6419 | 18254 | 30181 | 42084 | 55379 |
| 20 | 10 | 0 | -515 | -207 | -229 | -296 | -280 | -250 | -143 | 873 | 2570 | 5009 | 9498 | 54009 |
| 20 | 11 | 0 | -485 | -180 | -189 | -204 | -119 | 366 | 2936 | 11876 | 23537 | 33572 | 46748 | 54213 |
| 20 | 12 | 0 | -491 | -158 | -137 | -12 | 278 | 1084 | 2952 | 6513 | 11433 | 17705 | 28796 | 53893 |
| 20 | 13 | 0 | -519 | -156 | -156 | -217 | -143 | 76 | 687 | 2674 | 8469 | 17793 | 31112 | 55119 |
| 20 | 14 | 0 | -489 | -156 | -186 | -238 | -186 | 64 | 784 | 2255 | 4730 | 8671 | 19777 | 52854 |
| 20 | 15 | 0 | -552 | -186 | -195 | -250 | -168 | -46 | 315 | 2787 | 10084 | 27483 | 44868 | 53386 |
| 20 | 16 | 0 | -534 | -210 | -238 | -277 | -226 | -189 | 40 | 1297 | 4990 | 13963 | 31393 | 50831 |
| 20 | 17 | 0 | -482 | -202 | -205 | -205 | 52 | 1352 | 7221 | 15999 | 30651 | 40900 | 50590 | 55482 |
| 20 | 18 | 0 | -519 | -207 | -134 | 98 | 724 | 2408 | 7282 | 15431 | 28259 | 39762 | 49397 | 55043 |
| 20 | 19 | 0 | -492 | -190 | -220 | -272 | -260 | -235 | -257 | -211 | -232 | -229 | -202 | 53330 |
| 20 | 20 | 0 | -461 | -137 | -79 | 92 | 601 | 1596 | 3788 | 9284 | 16505 | 25319 | 38162 | 54545 |
| 20 | 21 | 0 | -522 | -165 | -183 | -229 | -226 | -211 | -235 | -229 | -238 | -177 | -235 | -171 |
| 20 | 22 | 0 | -519 | -180 | -152 | -30 | 354 | 1172 | 3202 | 7819 | 18990 | 31207 | 43625 | 54149 |
| 20 | 23 | 0 | -522 | -159 | -189 | -244 | -159 | 201 | 995 | 2878 | 5255 | 8429 | 17329 | 53758 |
| 20 | 24 | 0 | -543 | -202 | -226 | -281 | -241 | -250 | -229 | 98 | 629 | 2637 | 12202 | 52580 |
| 20 | 25 | 0 | -448 | -168 | -30 | 223 | 837 | 2030 | 4505 | 7322 | 12102 | 16481 | 22823 | 54781 |
| 20 | 26 | 0 | -516 | -207 | -189 | -131 | 415 | 2619 | 8619 | 18507 | 33136 | 44691 | 50389 | 55131 |
| 20 | 27 | 0 | -494 | -183 | -222 | -247 | -213 | -219 | -231 | -183 | -125 | 28 | 998 | 54537 |
| 20 | 28 | 0 | -488 | -153 | -146 | -131 | 107 | 507 | 1511 | 3855 | 6696 | 10340 | 14558 | 53773 |
| 20 | 29 | 0 | -516 | -171 | -177 | -217 | -232 | -235 | -183 | 3 | 244 | 714 | 1581 | 53276 |
| 20 | 30 | 0 | -531 | -159 | -186 | -232 | -156 | 6 | 357 | 1340 | 2957 | 5020 | 8429 | 53938 |
| 20 | 31 | 0 | -558 | -186 | -189 | -27 | 766 | 2524 | 5454 | 11396 | 22399 | 34817 | 46879 | 53886 |
| 20 | 32 | 0 | -540 | -196 | -186 | -266 | -193 | -98 | 281 | 1684 | 4120 | 9031 | 22441 | 51334 |
| 10 | 1 | 0 | -467 | -125 | 116 | 574 | 1340 | 2744 | 5512 | 8592 | 14656 | 23458 | 36939 | 55251 |
| 10 | 2 | 0 | -516 | -241 | -250 | -263 | -269 | -247 | -247 | -259 | -296 | -235 | -247 | -229 |
| 10 | 3 | 0 | -473 | -187 | -205 | -235 | -232 | -229 | -229 | -220 | -248 | -251 | -257 | -171 |
| 10 | 4 | 0 | -494 | -155 | -165 | -201 | -110 | 357 | 3254 | 11424 | 21615 | 31640 | 43494 | 55290 |
| 10 | 5 | 0 | -501 | -107 | -147 | -192 | -174 | -165 | -174 | -153 | -183 | -168 | -186 | -128 |
| 10 | 6 | 0 | -528 | -208 | -193 | -251 | -217 | -238 | -251 | -232 | -251 | -180 | -269 | -232 |
| 10 | 7 | 0 | -522 | -171 | -156 | 73 | 1193 | 3626 | 7523 | 14912 | 24361 | 34622 | 45774 | 52863 |
| 10 | 8 | 0 | -577 | -186 | -241 | -296 | -256 | -256 | -272 | -49 | 177 | 1147 | 5881 | 50196 |
| 10 | 9 | 0 | -470 | -202 | -168 | 67 | 1025 | 3045 | 6860 | 10730 | 16453 | 20518 | 25618 | 54295 |
| 10 | 10 | 0 | -489 | -211 | -223 | -244 | -263 | -251 | -257 | -269 | -290 | -257 | -275 | -183 |
| 10 | 11 | 0 | -488 | -183 | -198 | -244 | -198 | -183 | -183 | 113 | 702 | 1972 | 4880 | 53529 |
| 10 | 12 | 0 | -494 | -192 | -180 | -229 | -220 | -198 | -226 | -189 | -250 | -226 | -250 | -189 |
| 10 | 13 | 0 | -494 | -149 | -167 | -204 | -198 | -198 | -201 | -207 | -216 | -192 | -225 | -247 |
| 10 | 14 | 0 | -531 | -186 | -171 | -266 | -198 | -201 | -229 | -198 | -241 | -223 | -256 | -259 |
| 10 | 15 | 0 | -534 | -180 | -198 | -235 | -201 | -210 | -219 | -119 | -235 | -241 | -256 | -189 |
| 10 | 16 | 0 | -550 | -61 | -199 | -275 | -226 | -180 | -77 | 598 | 2453 | 7849 | 27107 | 51258 |
| 10 | 17 | 0 | -513 | -226 | -254 | -260 | -180 | 198 | 2841 | 9907 | 23042 | 32397 | 43451 | 54704 |
| 10 | 18 | 0 | -494 | -235 | -241 | -290 | -256 | -244 | -265 | -262 | -286 | -262 | -311 | -228 |
| 10 | 19 | 0 | -479 | -207 | -207 | -241 | -250 | -211 | -253 | -229 | -275 | -229 | -269 | -238 |
| 10 | 20 | 0 | -512 | -204 | -198 | -256 | -241 | -213 | -244 | -228 | -274 | -265 | -308 | -244 |
| 10 | 21 | 0 | -521 | -155 | -192 | -219 | -201 | -210 | -222 | -210 | -241 | -183 | -271 | -244 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*C. freundii* FRHCFR2 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -501 | -150 | -58 | 183 | 662 | 1520 | 2679 | 5087 | 8445 | 13325 | 19883 | 53165 |
| 10 | 23 | 0 | -528 | -171 | -110 | 296 | 1272 | 3198 | 6336 | 12607 | 21419 | 31130 | 43494 | 52967 |
| 10 | 24 | 0 | -571 | -92 | -253 | -308 | -265 | -296 | -317 | -217 | -348 | -330 | -388 | -333 |
| 10 | 25 | 0 | -501 | -232 | -272 | -287 | -272 | -278 | -293 | -253 | -226 | -293 | -296 | -229 |
| 10 | 26 | 0 | -482 | -213 | -222 | -271 | -247 | -238 | -247 | -235 | -287 | -235 | -290 | -207 |
| 10 | 27 | 0 | -491 | -208 | -201 | -232 | -147 | 269 | 3153 | 9891 | 21077 | 30584 | 41306 | 54017 |
| 10 | 28 | 0 | -534 | -220 | -220 | -278 | -220 | -226 | -260 | -232 | -293 | -223 | -303 | -257 |
| 10 | 29 | 0 | -562 | -214 | -223 | -263 | -223 | -214 | -241 | -214 | -241 | -208 | -287 | -253 |
| 10 | 30 | 0 | -544 | -180 | -193 | -199 | -25 | 695 | 2850 | 9720 | 20198 | 30825 | 43326 | 53675 |
| 10 | 31 | 0 | -534 | -202 | -223 | -250 | -211 | -232 | -244 | -156 | -263 | -250 | -253 | -186 |
| 10 | 32 | 0 | -568 | -95 | -229 | -290 | -250 | -247 | -263 | -131 | -180 | -73 | 171 | 50410 |
| 5 | 1 | 0 | -498 | -220 | -235 | -101 | 506 | 1590 | 5521 | 14054 | 26326 | 34612 | 44516 | 55543 |
| 5 | 2 | 0 | -452 | -183 | -198 | -229 | -198 | -220 | -232 | -202 | -253 | -226 | -263 | -192 |
| 5 | 3 | 0 | -503 | -198 | -226 | -268 | -268 | -238 | -262 | -226 | -272 | -262 | -305 | -278 |
| 5 | 4 | 0 | -510 | -207 | -201 | -253 | -186 | -232 | -235 | -232 | -265 | -201 | -268 | -217 |
| 5 | 5 | 0 | -540 | -208 | -220 | -269 | -229 | -217 | -254 | -214 | -250 | -202 | -305 | -235 |
| 5 | 6 | 0 | -513 | -190 | -190 | -242 | -214 | -211 | -220 | -211 | -232 | -177 | -269 | -217 |
| 5 | 7 | 0 | -534 | -201 | -210 | -247 | -167 | -149 | 415 | 2247 | 5027 | 8845 | 17461 | 52531 |
| 5 | 8 | 0 | -561 | -70 | -250 | -259 | -174 | -30 | 510 | 2030 | 5369 | 16667 | 39878 | 51576 |
| 5 | 9 | 0 | -500 | -238 | -268 | -287 | -287 | -284 | -287 | -259 | -323 | -302 | -333 | -293 |
| 5 | 10 | 0 | -501 | -220 | -235 | -293 | -247 | -238 | -260 | -226 | -305 | -250 | -311 | -229 |
| 5 | 11 | 0 | -513 | -235 | -241 | -305 | -284 | -263 | -284 | -229 | -281 | -266 | -312 | -260 |
| 5 | 12 | 0 | -535 | -217 | -229 | -242 | -77 | 329 | 3152 | 11130 | 22249 | 31823 | 42196 | 55384 |
| 5 | 13 | 0 | -534 | -232 | -220 | -287 | -235 | -235 | -259 | -250 | -256 | -216 | -293 | -223 |
| 5 | 14 | 0 | -531 | -195 | -214 | -241 | -220 | -205 | -250 | -217 | -260 | -189 | -275 | -247 |
| 5 | 15 | 0 | -564 | -217 | -247 | -278 | -244 | -259 | -265 | -177 | -293 | -290 | -317 | -247 |
| 5 | 16 | 0 | -574 | -70 | -244 | -204 | 52 | 806 | 2210 | 5103 | 9226 | 16545 | 38907 | 51909 |
| 5 | 17 | 0 | -492 | -223 | -248 | -290 | -254 | -275 | -281 | -241 | -272 | -287 | -269 | -214 |
| 5 | 18 | 0 | -510 | -186 | -101 | 354 | 1288 | 2689 | 4108 | 7355 | 12147 | 18907 | 27538 | 54979 |
| 5 | 19 | 0 | -504 | -202 | -229 | -263 | -239 | -181 | -153 | 112 | 1065 | 3375 | 10367 | 54325 |
| 5 | 20 | 0 | -540 | -220 | -265 | -281 | -217 | -275 | -253 | -241 | -278 | -226 | -269 | -189 |
| 5 | 21 | 0 | -528 | -183 | -204 | -265 | -228 | -216 | -235 | -225 | -250 | -198 | -274 | -192 |
| 5 | 22 | 0 | -540 | -192 | -189 | -235 | -183 | -46 | 384 | 1801 | 5271 | 10752 | 20604 | 54442 |
| 5 | 23 | 0 | -543 | -211 | -223 | -272 | -217 | -259 | -250 | -180 | -272 | -290 | -299 | -211 |
| 5 | 24 | 0 | -595 | -74 | -257 | -318 | -290 | -272 | -305 | -247 | -336 | -351 | -357 | -305 |
| 5 | 25 | 0 | -497 | -211 | -241 | -192 | 205 | 1914 | 6165 | 15117 | 24950 | 33892 | 43705 | 56547 |
| 5 | 26 | 0 | -537 | -247 | -281 | -311 | -281 | -281 | -302 | -265 | -293 | -287 | -323 | -241 |
| 5 | 27 | 0 | -497 | -229 | -253 | -317 | -274 | -284 | -302 | -259 | -339 | -268 | -317 | -256 |
| 5 | 28 | 0 | -531 | -226 | -272 | -299 | -250 | -269 | -272 | -235 | -311 | -244 | -333 | -208 |
| 5 | 29 | 0 | -528 | -177 | -198 | -244 | -170 | -21 | 382 | 1127 | 2146 | 3187 | 4520 | 55840 |
| 5 | 30 | 0 | -556 | -217 | -241 | -269 | -245 | -248 | -263 | -241 | -272 | -238 | -290 | -150 |
| 5 | 31 | 0 | -522 | -144 | 0 | 238 | 409 | 1071 | 1712 | 3763 | 7523 | 11601 | 16847 | 54164 |
| 5 | 32 | 0 | -528 | -12 | -220 | -281 | -250 | -241 | -266 | -156 | -296 | -275 | -327 | -241 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*K. pneumoniae* FRHKPC2 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -180 | -302 | -238 | -265 | -42 | 528 | 3672 | 13740 | 24087 | 29934 | 34924 | 42338 |
| 20 | 2 | 0 | -196 | -306 | -235 | -315 | -214 | -220 | -126 | 1251 | 7755 | 15815 | 24635 | 42770 |
| 20 | 3 | 0 | -192 | -311 | -244 | -265 | -186 | -110 | 293 | 4423 | 11784 | 17595 | 23626 | 42646 |
| 20 | 4 | 0 | -168 | -314 | -238 | -232 | -161 | -100 | 174 | 3391 | 11869 | 18761 | 24978 | 42557 |
| 20 | 5 | 0 | -165 | -305 | -241 | -278 | -220 | -149 | 159 | 3669 | 13432 | 21221 | 27740 | 42569 |
| 20 | 6 | 0 | -207 | -351 | -308 | -308 | -275 | -229 | 363 | 7804 | 18800 | 24807 | 29931 | 42753 |
| 20 | 7 | 0 | -174 | -299 | -265 | -244 | -186 | 58 | 1557 | 11308 | 20882 | 25826 | 30673 | 42353 |
| 20 | 8 | 0 | -143 | -287 | -247 | -262 | -168 | -104 | 455 | 5326 | 14656 | 21196 | 29153 | 42124 |
| 20 | 9 | 0 | -211 | -311 | -250 | -305 | -174 | -55 | 827 | 8387 | 17396 | 23006 | 28420 | 43128 |
| 20 | 10 | 0 | -205 | -312 | -260 | -321 | -244 | -284 | -397 | -202 | 110 | 1227 | 5792 | 42255 |
| 20 | 11 | 0 | -192 | -302 | -256 | -256 | -189 | -186 | -100 | 1010 | 6492 | 13045 | 20720 | 42240 |
| 20 | 12 | 0 | -131 | -287 | -195 | -220 | -162 | -165 | 272 | 4505 | 13642 | 20854 | 27233 | 42670 |
| 20 | 13 | 0 | -140 | -299 | -244 | -284 | -210 | -204 | -158 | 1020 | 6388 | 13112 | 21148 | 42277 |
| 20 | 14 | 0 | -153 | -284 | -235 | -272 | -220 | -266 | -345 | -131 | 378 | 2457 | 9385 | 42032 |
| 20 | 15 | 0 | -156 | -305 | -244 | -250 | -232 | -278 | -357 | -201 | -211 | -214 | -159 | -171 |
| 20 | 16 | 0 | -171 | -284 | -257 | -269 | -159 | 149 | 1520 | 8976 | 16630 | 21208 | 25566 | 42654 |
| 20 | 17 | 0 | -193 | -299 | -244 | -296 | -189 | -116 | 238 | 3741 | 12431 | 19935 | 27025 | 43115 |
| 20 | 18 | 0 | -165 | -268 | -207 | -268 | -149 | -82 | 556 | 5140 | 14119 | 21038 | 27401 | 42637 |
| 20 | 19 | 0 | -192 | -296 | -229 | -250 | -201 | -195 | -18 | 1807 | 7990 | 14885 | 22838 | 42454 |
| 20 | 20 | 0 | -158 | -280 | -247 | -244 | -201 | -235 | -277 | 92 | 1175 | 3962 | 10737 | 42454 |
| 20 | 21 | 0 | -138 | -263 | -202 | -244 | -177 | -217 | -241 | 143 | 1187 | 4352 | 12003 | 42194 |
| 20 | 22 | 0 | -122 | -272 | -214 | -223 | -131 | -15 | 610 | 5237 | 12498 | 17790 | 23122 | 42581 |
| 20 | 23 | 0 | -165 | -308 | -214 | -241 | -223 | -201 | -86 | 1248 | 5668 | 11555 | 18062 | 41950 |
| 20 | 24 | 0 | -152 | -284 | -250 | -259 | -189 | -137 | 122 | 2735 | 9544 | 15831 | 21694 | 41987 |
| 20 | 25 | 0 | -208 | -342 | -244 | -293 | -232 | -192 | 128 | 3336 | 11134 | 18675 | 25612 | 42758 |
| 20 | 26 | 0 | -152 | -272 | -217 | -265 | -183 | -192 | -101 | 891 | 4804 | 10728 | 18849 | 42298 |
| 20 | 27 | 0 | -162 | -284 | -226 | -232 | -202 | -242 | -281 | 6 | 512 | 1846 | 6430 | 42605 |
| 20 | 28 | 0 | -174 | -284 | -199 | -239 | -214 | -242 | -239 | 164 | 1129 | 3268 | 9140 | 42468 |
| 20 | 29 | 0 | -146 | -268 | -210 | -241 | -189 | -189 | -159 | 644 | 2927 | 6531 | 12315 | 42264 |
| 20 | 30 | 0 | -153 | -275 | -223 | -263 | -223 | -238 | -263 | 27 | 696 | 2371 | 7129 | 41745 |
| 20 | 31 | 0 | -144 | -287 | -211 | -220 | -186 | -134 | 36 | 1672 | 5732 | 10520 | 16520 | 41800 |
| 20 | 32 | 0 | -150 | -281 | -208 | -250 | -186 | -174 | -40 | 1572 | 5591 | 10160 | 14766 | 41876 |
| 10 | 1 | 0 | -202 | -315 | -229 | -299 | -223 | -235 | -220 | 177 | 1218 | 4022 | 11539 | 42502 |
| 10 | 2 | 0 | -149 | -259 | -201 | -262 | -177 | -214 | -247 | 104 | 998 | 3168 | 8994 | 42151 |
| 10 | 3 | 0 | -156 | -251 | -196 | -214 | -174 | -217 | -269 | -19 | 424 | 1345 | 5212 | 41745 |
| 10 | 4 | 0 | -144 | -263 | -208 | -248 | -220 | -248 | -327 | -229 | -165 | -129 | 122 | 42590 |
| 10 | 5 | 0 | -122 | -250 | -183 | -244 | -180 | -211 | -302 | -107 | 89 | 504 | 2115 | 42145 |
| 10 | 6 | 0 | -116 | -281 | -220 | -259 | -211 | -253 | -323 | -149 | 119 | 760 | 2960 | 41739 |
| 10 | 7 | 0 | -125 | -263 | -193 | -196 | -177 | -214 | -196 | 409 | 1803 | 4767 | 10007 | 41653 |
| 10 | 8 | 0 | -156 | -275 | -223 | -275 | -223 | -284 | -366 | -195 | -229 | -226 | -189 | -195 |
| 10 | 9 | 0 | -177 | -290 | -208 | -269 | -180 | -180 | -16 | 1437 | 5969 | 11905 | 20854 | 42828 |
| 10 | 10 | 0 | -156 | -275 | -198 | -262 | -192 | -253 | -345 | -235 | -180 | -189 | -159 | -168 |
| 10 | 11 | 0 | -137 | -238 | -171 | -202 | -162 | -208 | -302 | -122 | 55 | 424 | 2396 | 41992 |
| 10 | 12 | 0 | -119 | -220 | -171 | -189 | -149 | -217 | -284 | -171 | -143 | -149 | -98 | 42051 |
| 10 | 13 | 0 | -89 | -214 | -150 | -192 | -147 | -180 | -272 | -141 | -125 | -95 | 101 | 42066 |
| 10 | 14 | 0 | -107 | -235 | -177 | -195 | -162 | -220 | -311 | -177 | -146 | -122 | 58 | 42139 |
| 10 | 15 | 0 | -128 | -266 | -180 | -208 | -180 | -232 | -302 | -77 | 76 | 522 | 2341 | 41489 |
| 10 | 16 | 0 | -141 | -269 | -217 | -253 | -171 | -131 | -67 | 586 | 1904 | 4697 | 9864 | 41724 |
| 10 | 17 | 0 | -171 | -290 | -219 | -262 | -210 | -265 | -271 | 214 | 1478 | 5073 | 15556 | 42567 |
| 10 | 18 | 0 | -186 | -305 | -216 | -296 | -222 | -256 | -335 | -235 | -33 | 290 | 1804 | 41810 |
| 10 | 19 | 0 | -122 | -260 | -183 | -208 | -168 | -195 | -302 | -125 | 30 | 250 | 1242 | 41824 |
| 10 | 20 | 0 | -101 | -229 | -171 | -207 | -143 | -195 | -253 | -146 | -137 | -79 | 180 | 42246 |
| 10 | 21 | 0 | -64 | -186 | -146 | -164 | -140 | -161 | -250 | -119 | -103 | -85 | 68 | 41944 |

**Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.**

*K. pneumoniae* FRHKPC2 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -104 | -253 | -186 | -213 | -165 | -219 | -293 | -165 | -165 | -146 | -91 | 42017 |
| 10 | 23 | 0 | -125 | -244 | -202 | -229 | -171 | -241 | -293 | -95 | -40 | 55 | 299 | 40787 |
| 10 | 24 | 0 | -143 | -274 | -223 | -262 | -201 | -189 | -55 | 1215 | 4420 | 8671 | 14921 | 41898 |
| 10 | 25 | 0 | -168 | -275 | -223 | -256 | -171 | -174 | 31 | 1718 | 6531 | 12632 | 20973 | 42518 |
| 10 | 26 | 0 | -156 | -266 | -183 | -259 | -192 | -208 | -262 | 34 | 775 | 2576 | 7706 | 41910 |
| 10 | 27 | 0 | -137 | -260 | -186 | -199 | -165 | -214 | -293 | -153 | -40 | 168 | 976 | 41675 |
| 10 | 28 | 0 | -100 | -262 | -164 | -222 | -161 | -216 | -271 | -146 | -61 | 138 | 861 | 42267 |
| 10 | 29 | 0 | -101 | -248 | -199 | -217 | -153 | -193 | -287 | -208 | -107 | -64 | 265 | 41626 |
| 10 | 30 | 0 | -128 | -260 | -208 | -232 | -195 | -238 | -302 | -174 | -195 | -199 | -153 | -162 |
| 10 | 31 | 0 | -113 | -247 | -217 | -235 | -174 | -220 | -217 | 268 | 1291 | 3543 | 8225 | 41742 |
| 10 | 32 | 0 | -128 | -269 | -214 | -256 | -198 | -247 | -269 | 122 | 885 | 3089 | 8442 | 41638 |
| 5 | 1 | 0 | -168 | -278 | -232 | -262 | -216 | -274 | -354 | -244 | -201 | -220 | -189 | -210 |
| 5 | 2 | 0 | -174 | -293 | -170 | -247 | -186 | -219 | -314 | -238 | -170 | -195 | -164 | -186 |
| 5 | 3 | 0 | -143 | -275 | -211 | -208 | -177 | -220 | -321 | -192 | -125 | 49 | 848 | 41644 |
| 5 | 4 | 0 | -131 | -265 | -186 | -210 | -189 | -253 | -323 | -177 | -180 | -195 | -149 | -155 |
| 5 | 5 | 0 | -95 | -235 | -187 | -232 | -177 | -184 | -278 | -156 | -16 | 88 | 467 | 41495 |
| 5 | 6 | 0 | -134 | -266 | -220 | -241 | -189 | -238 | -284 | -15 | 253 | 757 | 2509 | 41730 |
| 5 | 7 | 0 | -153 | -278 | -238 | -272 | -205 | -257 | -348 | -192 | -214 | -195 | -165 | -208 |
| 5 | 8 | 0 | -140 | -287 | -223 | -247 | -183 | -137 | 122 | 2017 | 6305 | 11094 | 17445 | 41883 |
| 5 | 9 | 0 | -137 | -266 | -198 | -256 | -180 | -220 | -247 | 3 | 290 | 949 | 5075 | 42282 |
| 5 | 10 | 0 | -164 | -286 | -216 | -274 | -210 | -250 | -329 | -207 | 40 | 559 | 2885 | 42188 |
| 5 | 11 | 0 | -146 | -274 | -213 | -223 | -198 | -195 | -320 | -140 | 3 | 232 | 1239 | 41843 |
| 5 | 12 | 0 | -152 | -286 | -222 | -247 | -192 | -271 | -354 | -204 | -219 | -219 | -173 | -183 |
| 5 | 13 | 0 | -159 | -266 | -199 | -263 | -165 | -217 | -275 | -49 | 262 | 912 | 3494 | 42011 |
| 5 | 14 | 0 | -134 | -263 | -217 | -232 | -189 | -241 | -308 | -122 | 21 | 372 | 1984 | 42233 |
| 5 | 15 | 0 | -156 | -293 | -241 | -269 | -202 | -263 | -296 | -116 | -70 | 30 | 210 | 40784 |
| 5 | 16 | 0 | -153 | -293 | -269 | -284 | -223 | -272 | -360 | -198 | -235 | -226 | -186 | -205 |
| 5 | 17 | 0 | -159 | -290 | -201 | -272 | -192 | -250 | -342 | -238 | -198 | -207 | -174 | -192 |
| 5 | 18 | 0 | -177 | -293 | -223 | -272 | -193 | -174 | 18 | 1785 | 8408 | 15043 | 23582 | 43228 |
| 5 | 19 | 0 | -186 | -305 | -222 | -213 | -189 | -219 | -326 | -216 | -189 | -201 | -164 | -195 |
| 5 | 20 | 0 | -153 | -266 | -211 | -257 | -196 | -229 | -275 | 33 | 604 | 1996 | 7788 | 42493 |
| 5 | 21 | 0 | -137 | -278 | -195 | -229 | -183 | -189 | -229 | -6 | 287 | 537 | 1166 | 41904 |
| 5 | 22 | 0 | -134 | -287 | -211 | -244 | -186 | -244 | -324 | -180 | -180 | -195 | -159 | -143 |
| 5 | 23 | 0 | -168 | -296 | -232 | -262 | -201 | -192 | 86 | 2506 | 7932 | 13295 | 19362 | 41788 |
| 5 | 24 | 0 | -162 | -324 | -248 | -257 | -223 | -205 | -150 | 329 | 1495 | 4889 | 10773 | 42053 |
| 5 | 25 | 0 | -170 | -283 | -222 | -268 | -177 | -195 | -94 | 831 | 4456 | 9755 | 16738 | 42780 |
| 5 | 26 | 0 | -177 | -281 | -220 | -287 | -239 | -278 | -361 | -269 | -220 | -235 | -190 | -220 |
| 5 | 27 | 0 | -199 | -330 | -247 | -269 | -226 | -278 | -376 | -302 | -244 | -269 | -229 | -238 |
| 5 | 28 | 0 | -183 | -293 | -241 | -284 | -232 | -272 | -376 | -247 | -223 | -241 | -189 | -189 |
| 5 | 29 | 0 | -140 | -268 | -219 | -259 | -195 | -213 | -262 | -70 | 159 | 376 | 962 | 42139 |
| 5 | 30 | 0 | -155 | -299 | -250 | -271 | -219 | -253 | -332 | -113 | 52 | 608 | 4191 | 42619 |
| 5 | 31 | 0 | -168 | -296 | -259 | -278 | -235 | -281 | -366 | -198 | -235 | -241 | -195 | -204 |
| 5 | 32 | 0 | -164 | -326 | -283 | -290 | -262 | -305 | -372 | -235 | -253 | -277 | -222 | -241 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. cloacae* FRHECL2 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -260 | -370 | -504 | -455 | -159 | 381 | 1504 | 4355 | 10377 | 14347 | 23537 | 47562 |
| 20 | 2 | 0 | -260 | -360 | -513 | -437 | -186 | 287 | 2014 | 5109 | 10941 | 14201 | 21431 | 48029 |
| 20 | 3 | 0 | -305 | -342 | -482 | -409 | -210 | 327 | 1700 | 4584 | 10255 | 14424 | 19820 | 47581 |
| 20 | 4 | 0 | -300 | -370 | -470 | -388 | -49 | 778 | 3723 | 7819 | 13416 | 17198 | 23418 | 47400 |
| 20 | 5 | 0 | -293 | -339 | -461 | -425 | -168 | 390 | 1608 | 4440 | 9360 | 13138 | 22157 | 47345 |
| 20 | 6 | 0 | -281 | -361 | -504 | -443 | -303 | 30 | 366 | 1504 | 4431 | 8371 | 16071 | 47132 |
| 20 | 7 | 0 | -278 | -348 | -455 | -403 | -290 | 104 | 473 | 1401 | 3907 | 7160 | 14290 | 46449 |
| 20 | 8 | 0 | -283 | -378 | -497 | -436 | -286 | 138 | 568 | 1661 | 4896 | 8769 | 16793 | 46498 |
| 20 | 9 | 0 | -269 | -360 | -498 | -476 | -266 | 85 | 748 | 2603 | 7413 | 10410 | 16465 | 48228 |
| 20 | 10 | 0 | -265 | -338 | -461 | -412 | -171 | 315 | 1856 | 4636 | 10679 | 13921 | 19631 | 48002 |
| 20 | 11 | 0 | -290 | -330 | -452 | -443 | -287 | -55 | 287 | 1282 | 5820 | 9602 | 14552 | 47578 |
| 20 | 12 | 0 | -293 | -348 | -467 | -422 | -248 | 134 | 937 | 3268 | 8127 | 12012 | 17787 | 48053 |
| 20 | 13 | 0 | -274 | -363 | -452 | -439 | -244 | 162 | 1059 | 3409 | 8589 | 11982 | 18239 | 47572 |
| 20 | 14 | 0 | -281 | -323 | -455 | -424 | -308 | -128 | 92 | 733 | 4026 | 7734 | 14210 | 47324 |
| 20 | 15 | 0 | -259 | -336 | -437 | -382 | -269 | 61 | 458 | 2011 | 6879 | 10645 | 16676 | 47422 |
| 20 | 16 | 0 | -253 | -354 | -455 | -427 | -262 | 202 | 809 | 2503 | 6635 | 10215 | 16109 | 47276 |
| 20 | 17 | 0 | -278 | -375 | -510 | -473 | -238 | 165 | 1050 | 3327 | 8619 | 11167 | 17235 | 48231 |
| 20 | 18 | 0 | -272 | -357 | -495 | -461 | -327 | -159 | 24 | 583 | 3485 | 6629 | 11399 | 48224 |
| 20 | 19 | 0 | -290 | -342 | -467 | -436 | -259 | 37 | 550 | 2146 | 6361 | 10020 | 15620 | 47669 |
| 20 | 20 | 0 | -327 | -354 | -498 | -428 | -287 | -3 | 451 | 1904 | 6174 | 9440 | 14936 | 47968 |
| 20 | 21 | 0 | -262 | -351 | -455 | -436 | -302 | -61 | 250 | 1361 | 5210 | 8286 | 13890 | 47837 |
| 20 | 22 | 0 | -265 | -332 | -448 | -452 | -281 | 6 | 431 | 1651 | 6016 | 9382 | 14683 | 47831 |
| 20 | 23 | 0 | -274 | -345 | -470 | -442 | -302 | 40 | 528 | 1917 | 6913 | 11021 | 16328 | 47514 |
| 20 | 24 | 0 | -259 | -342 | -448 | -409 | -265 | 134 | 766 | 2427 | 6882 | 10035 | 16054 | 47202 |
| 20 | 25 | 0 | -253 | -347 | -473 | -463 | -289 | -18 | 260 | 1304 | 4994 | 8012 | 14107 | 47899 |
| 20 | 26 | 0 | -235 | -357 | -501 | -449 | -312 | -141 | 27 | 686 | 3686 | 6867 | 11475 | 47971 |
| 20 | 27 | 0 | -281 | -348 | -483 | -467 | -315 | -122 | 73 | 693 | 3390 | 6021 | 11210 | 47809 |
| 20 | 28 | 0 | -244 | -311 | -440 | -403 | -278 | -43 | 204 | 1032 | 4001 | 6788 | 12193 | 47535 |
| 20 | 29 | 0 | -241 | -318 | -452 | -437 | -311 | -128 | -49 | 372 | 1953 | 4123 | 8564 | 47239 |
| 20 | 30 | 0 | -269 | -324 | -434 | -440 | -263 | 6 | 470 | 2008 | 5738 | 8903 | 14405 | 47458 |
| 20 | 31 | 0 | -271 | -366 | -452 | -461 | -357 | -119 | 22 | 763 | 3843 | 7197 | 12297 | 47218 |
| 20 | 32 | 0 | -268 | -360 | -457 | -403 | -287 | 98 | 879 | 2665 | 6654 | 10084 | 15740 | 47145 |
| 10 | 1 | 0 | -257 | -333 | -483 | -446 | -306 | -226 | -168 | 146 | 1126 | 3003 | 8914 | 47907 |
| 10 | 2 | 0 | -198 | -321 | -449 | -424 | -293 | -223 | -202 | 18 | 525 | 1852 | 4923 | 47480 |
| 10 | 3 | 0 | -265 | -326 | -470 | -418 | -342 | -250 | -333 | -217 | -94 | 223 | 1773 | 46964 |
| 10 | 4 | 0 | -238 | -308 | -439 | -430 | -296 | -232 | -293 | -195 | -91 | 220 | 1773 | 46827 |
| 10 | 5 | 0 | -229 | -342 | -467 | -461 | -342 | -211 | -171 | 61 | 781 | 2295 | 5997 | 47065 |
| 10 | 6 | 0 | -287 | -339 | -449 | -431 | -342 | -244 | -311 | -244 | -144 | 88 | 1681 | 47095 |
| 10 | 7 | 0 | -247 | -348 | -449 | -437 | -333 | -241 | -220 | -15 | 690 | 2182 | 5789 | 46787 |
| 10 | 8 | 0 | -265 | -345 | -488 | -439 | -351 | -253 | -241 | 9 | 867 | 3028 | 6858 | 47007 |
| 10 | 9 | 0 | -242 | -327 | -486 | -452 | -327 | -245 | -211 | 18 | 665 | 2087 | 5985 | 48026 |
| 10 | 10 | 0 | -210 | -308 | -442 | -442 | -308 | -241 | -299 | -195 | -55 | 290 | 1764 | 47660 |
| 10 | 11 | 0 | -205 | -290 | -415 | -382 | -287 | -241 | -314 | -253 | -305 | -281 | 189 | 46915 |
| 10 | 12 | 0 | -189 | -287 | -433 | -415 | -281 | -223 | -305 | -259 | -336 | -342 | 119 | 46906 |
| 10 | 13 | 0 | -189 | -299 | -436 | -436 | -311 | -226 | -296 | -265 | -326 | -345 | 229 | 47099 |
| 10 | 14 | 0 | -250 | -348 | -449 | -397 | -321 | -232 | -241 | -95 | 284 | 1309 | 4422 | 47883 |
| 10 | 15 | 0 | -244 | -345 | -440 | -409 | -351 | -165 | -73 | 326 | 1859 | 3632 | 8033 | 46796 |
| 10 | 16 | 0 | -250 | -329 | -470 | -418 | -357 | -210 | -137 | 238 | 1447 | 3898 | 7896 | 47135 |
| 10 | 17 | 0 | -220 | -336 | -479 | -452 | -293 | -217 | -247 | -46 | 482 | 1715 | 4914 | 47230 |
| 10 | 18 | 0 | -180 | -277 | -442 | -424 | -296 | -229 | -305 | -247 | -317 | -329 | 80 | 47489 |
| 10 | 19 | 0 | -180 | -256 | -406 | -360 | -250 | -202 | -287 | -232 | -348 | -415 | -192 | 47050 |
| 10 | 20 | 0 | -156 | -251 | -391 | -345 | -251 | -181 | -257 | -220 | -358 | -440 | -174 | 46585 |
| 10 | 21 | 0 | -177 | -263 | -418 | -379 | -284 | -205 | -287 | -241 | -382 | -437 | -110 | 46650 |

**Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.**

*E. cloacae* FRHECL2 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -263 | -324 | -418 | -388 | -299 | -192 | -269 | -177 | -76 | 220 | 1730 | 47614 |
| 10 | 23 | 0 | -232 | -339 | -449 | -427 | -333 | -174 | -141 | 204 | 1370 | 3052 | 7142 | 46784 |
| 10 | 24 | 0 | -247 | -357 | -503 | -445 | -390 | -207 | -152 | 205 | 1566 | 4182 | 8390 | 47215 |
| 10 | 25 | 0 | -226 | -351 | -479 | -430 | -305 | -229 | -238 | -45 | 492 | 1554 | 4438 | 47245 |
| 10 | 26 | 0 | -168 | -269 | -440 | -397 | -287 | -223 | -302 | -247 | -324 | -430 | -76 | 47025 |
| 10 | 27 | 0 | -183 | -254 | -406 | -379 | -244 | -183 | -257 | -232 | -339 | -400 | -205 | 46558 |
| 10 | 28 | 0 | -159 | -226 | -400 | -342 | -257 | -174 | -248 | -205 | -379 | -428 | -171 | 46607 |
| 10 | 29 | 0 | -171 | -272 | -421 | -394 | -290 | -192 | -296 | -247 | -381 | -461 | -214 | 46818 |
| 10 | 30 | 0 | -241 | -311 | -421 | -384 | -302 | -213 | -283 | -201 | -149 | 10 | 1246 | 46531 |
| 10 | 31 | 0 | -232 | -312 | -455 | -403 | -330 | -186 | -150 | 125 | 1013 | 2505 | 6296 | 46546 |
| 10 | 32 | 0 | -256 | -354 | -457 | -436 | -372 | -155 | -27 | 510 | 2146 | 5033 | 9059 | 47114 |
| 5 | 1 | 0 | -241 | -344 | -500 | -470 | -314 | -274 | -341 | -271 | -225 | -143 | 699 | 47108 |
| 5 | 2 | 0 | -162 | -299 | -418 | -406 | -290 | -232 | -308 | -241 | -326 | -360 | 16 | 47486 |
| 5 | 3 | 0 | -226 | -293 | -442 | -424 | -283 | -210 | -308 | -262 | -387 | -439 | -222 | 47026 |
| 5 | 4 | 0 | -150 | -245 | -400 | -361 | -263 | -174 | -260 | -214 | -345 | -452 | -184 | 46869 |
| 5 | 5 | 0 | -186 | -275 | -440 | -391 | -302 | -223 | -311 | -290 | -397 | -485 | -214 | 46540 |
| 5 | 6 | 0 | -214 | -284 | -431 | -379 | -287 | -217 | -302 | -248 | -385 | -446 | -16 | 47004 |
| 5 | 7 | 0 | -253 | -351 | -485 | -445 | -348 | -216 | -207 | 28 | 785 | 2228 | 5967 | 46711 |
| 5 | 8 | 0 | -223 | -326 | -482 | -412 | -375 | -247 | -317 | -186 | -6 | 644 | 2869 | 47208 |
| 5 | 9 | 0 | -256 | -382 | -504 | -507 | -372 | -293 | -357 | -281 | -116 | 92 | 1700 | 47550 |
| 5 | 10 | 0 | -217 | -306 | -449 | -446 | -321 | -263 | -324 | -278 | -287 | -339 | 195 | 47373 |
| 5 | 11 | 0 | -189 | -278 | -436 | -391 | -278 | -214 | -296 | -244 | -354 | -439 | -253 | -79 |
| 5 | 12 | 0 | -205 | -278 | -421 | -385 | -293 | -217 | -293 | -247 | -403 | -455 | -137 | 47071 |
| 5 | 13 | 0 | -204 | -308 | -443 | -421 | -308 | -229 | -308 | -253 | -339 | -305 | 217 | 46796 |
| 5 | 14 | 0 | -250 | -335 | -476 | -430 | -342 | -253 | -320 | -247 | -174 | 6 | 1392 | 46998 |
| 5 | 15 | 0 | -244 | -327 | -464 | -418 | -351 | -238 | -305 | -229 | -192 | 15 | 1376 | 46747 |
| 5 | 16 | 0 | -235 | -339 | -449 | -458 | -369 | -244 | -311 | -232 | -137 | 278 | 1993 | 46546 |
| 5 | 17 | 0 | -278 | -360 | -489 | -480 | -336 | -238 | -196 | 146 | 1083 | 2789 | 6577 | 47309 |
| 5 | 18 | 0 | -222 | -311 | -464 | -436 | -314 | -250 | -338 | -262 | -244 | -201 | 486 | 47425 |
| 5 | 19 | 0 | -223 | -312 | -425 | -437 | -296 | -211 | -287 | -180 | -83 | 146 | 1684 | 47046 |
| 5 | 20 | 0 | -204 | -299 | -455 | -397 | -333 | -223 | -299 | -229 | -268 | -195 | 559 | 47153 |
| 5 | 21 | 0 | -226 | -320 | -445 | -442 | -308 | -238 | -323 | -302 | -427 | -473 | -189 | 47138 |
| 5 | 22 | 0 | -220 | -311 | -437 | -394 | -314 | -226 | -272 | -156 | -40 | 296 | 2170 | 47336 |
| 5 | 23 | 0 | -269 | -324 | -467 | -397 | -330 | -250 | -324 | -198 | -6 | 519 | 3244 | 46717 |
| 5 | 24 | 0 | -229 | -342 | -491 | -433 | -378 | -229 | -253 | -55 | 611 | 2466 | 6474 | 47141 |
| 5 | 25 | 0 | -293 | -364 | -510 | -489 | -342 | -275 | -315 | -153 | 131 | 659 | 3033 | 47128 |
| 5 | 26 | 0 | -219 | -314 | -445 | -445 | -302 | -238 | -287 | -177 | 55 | 385 | 2143 | 47831 |
| 5 | 27 | 0 | -247 | -308 | -476 | -457 | -317 | -198 | -235 | -61 | 321 | 1090 | 4209 | 47267 |
| 5 | 28 | 0 | -250 | -339 | -476 | -433 | -363 | -263 | -247 | -73 | 311 | 1581 | 4996 | 47553 |
| 5 | 29 | 0 | -238 | -335 | -467 | -430 | -323 | -256 | -290 | -174 | 77 | 711 | 3171 | 47196 |
| 5 | 30 | 0 | -238 | -351 | -479 | -405 | -347 | -247 | -265 | -45 | 556 | 2235 | 5250 | 47273 |
| 5 | 31 | 0 | -241 | -336 | -473 | -421 | -342 | -244 | -287 | -177 | -30 | 491 | 3232 | 46909 |
| 5 | 32 | 0 | -253 | -341 | -473 | -430 | -357 | -119 | 107 | 767 | 4887 | 8903 | 15300 | 46791 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. aerogenes* FRHEAE2 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -366 | -393 | -696 | -622 | -534 | -543 | -522 | -320 | -18 | 885 | 3602 | 48164 |
| 20 | 2 | 0 | -367 | -367 | -706 | -675 | -550 | -516 | -431 | -171 | 289 | 1449 | 5386 | 48123 |
| 20 | 3 | 0 | -388 | -394 | -724 | -675 | -602 | -599 | -632 | -498 | -498 | -193 | 857 | 49628 |
| 20 | 4 | 0 | -369 | -375 | -699 | -674 | -577 | -570 | -613 | -488 | -464 | -128 | 901 | 48521 |
| 20 | 5 | 0 | -372 | -394 | -690 | -653 | -592 | -583 | -610 | -503 | -458 | -46 | 1135 | 47413 |
| 20 | 6 | 0 | -460 | -494 | -784 | -772 | -711 | -702 | -738 | -689 | -714 | -589 | -128 | 45237 |
| 20 | 7 | 0 | -403 | -419 | -742 | -693 | -657 | -654 | -629 | -467 | -80 | 662 | 2182 | 43710 |
| 20 | 8 | 0 | -403 | -400 | -723 | -683 | -671 | -653 | -686 | -619 | -650 | -329 | 687 | 41828 |
| 20 | 9 | 0 | -379 | -388 | -714 | -650 | -565 | -580 | -556 | -434 | -333 | -64 | 1074 | 47767 |
| 20 | 10 | 0 | -379 | -369 | -720 | -675 | -559 | -559 | -516 | -366 | -287 | 183 | 1306 | 48313 |
| 20 | 11 | 0 | -339 | -348 | -669 | -620 | -516 | -510 | -519 | -367 | -263 | 140 | 1465 | 49100 |
| 20 | 12 | 0 | -351 | -357 | -683 | -653 | -546 | -531 | -488 | -332 | -219 | 354 | 2201 | 48295 |
| 20 | 13 | 0 | -354 | -363 | -680 | -634 | -558 | -500 | -442 | -207 | 315 | 1447 | 5164 | 47737 |
| 20 | 14 | 0 | -330 | -351 | -663 | -629 | -537 | -455 | -281 | 70 | 937 | 2542 | 7264 | 46134 |
| 20 | 15 | 0 | -354 | -357 | -671 | -629 | -580 | -561 | -580 | -470 | -439 | -259 | 229 | 46198 |
| 20 | 16 | 0 | -372 | -369 | -686 | -631 | -577 | -531 | -485 | -232 | 186 | 1184 | 4450 | 46403 |
| 20 | 17 | 0 | -382 | -370 | -672 | -620 | -541 | -507 | -422 | -238 | 30 | 665 | 2044 | 47998 |
| 20 | 18 | 0 | -363 | -363 | -702 | -659 | -549 | -549 | -546 | -402 | -369 | -155 | 504 | 48130 |
| 20 | 19 | 0 | -384 | -381 | -699 | -674 | -558 | -573 | -573 | -485 | -500 | -302 | 214 | 47868 |
| 20 | 20 | 0 | -348 | -351 | -674 | -653 | -525 | -500 | -430 | -256 | -36 | 919 | 2347 | 48310 |
| 20 | 21 | 0 | -342 | -375 | -659 | -604 | -540 | -546 | -571 | -485 | -488 | -290 | 266 | 48093 |
| 20 | 22 | 0 | -363 | -348 | -674 | -635 | -558 | -540 | -525 | -397 | -348 | -149 | 543 | 48530 |
| 20 | 23 | 0 | -327 | -318 | -629 | -605 | -531 | -528 | -541 | -464 | -443 | -290 | 192 | 46707 |
| 20 | 24 | 0 | -341 | -354 | -677 | -644 | -576 | -552 | -573 | -497 | -464 | -290 | 193 | 46763 |
| 20 | 25 | 0 | -348 | -381 | -683 | -631 | -558 | -546 | -506 | -351 | -277 | 107 | 1041 | 47709 |
| 20 | 26 | 0 | -381 | -375 | -698 | -653 | -534 | -534 | -494 | -329 | -219 | 208 | 1261 | 47786 |
| 20 | 27 | 0 | -351 | -348 | -684 | -632 | -543 | -531 | -552 | -452 | -458 | -259 | 156 | 47801 |
| 20 | 28 | 0 | -342 | -348 | -678 | -638 | -546 | -497 | -464 | -287 | -207 | 357 | 1734 | 47977 |
| 20 | 29 | 0 | -333 | -345 | -660 | -592 | -528 | -507 | -501 | -357 | -336 | -28 | 790 | 47409 |
| 20 | 30 | 0 | -357 | -360 | -659 | -617 | -546 | -510 | -495 | -342 | -211 | 67 | 1035 | 47477 |
| 20 | 31 | 0 | -336 | -342 | -653 | -617 | -556 | -531 | -522 | -388 | -336 | -159 | 583 | 47358 |
| 20 | 32 | 0 | -345 | -345 | -683 | -625 | -558 | -512 | -525 | -381 | -293 | -15 | 1065 | 47267 |
| 10 | 1 | 0 | -376 | -403 | -696 | -638 | -565 | -550 | -559 | -483 | -492 | -293 | 97 | 47303 |
| 10 | 2 | 0 | -391 | -400 | -708 | -675 | -568 | -583 | -586 | -455 | -409 | -235 | 384 | 47699 |
| 10 | 3 | 0 | -357 | -376 | -669 | -629 | -528 | -556 | -583 | -516 | -620 | -504 | -296 | 46952 |
| 10 | 4 | 0 | -321 | -345 | -657 | -602 | -525 | -504 | -556 | -458 | -528 | -376 | -86 | 47324 |
| 10 | 5 | 0 | -348 | -327 | -660 | -589 | -528 | -479 | -220 | 949 | 4077 | 7498 | 12900 | 47040 |
| 10 | 6 | 0 | -379 | -376 | -684 | -629 | -559 | -537 | -586 | -495 | -550 | -455 | -119 | 46576 |
| 10 | 7 | 0 | -324 | -339 | -620 | -623 | -537 | -541 | -562 | -501 | -531 | -431 | -138 | 46601 |
| 10 | 8 | 0 | -320 | -339 | -650 | -610 | -574 | -528 | -543 | -397 | -345 | -183 | 433 | 47187 |
| 10 | 9 | 0 | -369 | -396 | -689 | -628 | -546 | -558 | -577 | -424 | -418 | -207 | 446 | 47541 |
| 10 | 10 | 0 | -379 | -425 | -690 | -675 | -583 | -580 | -592 | -470 | -501 | -306 | 128 | 47864 |
| 10 | 11 | 0 | -357 | -384 | -705 | -659 | -580 | -558 | -543 | -403 | -372 | -91 | 913 | 47440 |
| 10 | 12 | 0 | -342 | -342 | -672 | -638 | -547 | -544 | -559 | -522 | -650 | -577 | -422 | 46878 |
| 10 | 13 | 0 | -332 | -348 | -662 | -573 | -519 | -509 | -519 | -360 | -323 | -100 | 574 | 47203 |
| 10 | 14 | 0 | -348 | -348 | -660 | -629 | -562 | -534 | -577 | -495 | -556 | -458 | -177 | 46726 |
| 10 | 15 | 0 | -308 | -314 | -622 | -598 | -540 | -516 | -537 | -430 | -397 | -299 | 104 | 46711 |
| 10 | 16 | 0 | -339 | -357 | -644 | -601 | -562 | -528 | -574 | -488 | -583 | -482 | -89 | 46451 |
| 10 | 17 | 0 | -339 | -360 | -656 | -613 | -537 | -537 | -540 | -421 | -427 | -214 | 391 | 47297 |
| 10 | 18 | 0 | -357 | -375 | -708 | -647 | -574 | -549 | -513 | -406 | -375 | -146 | 479 | 47682 |
| 10 | 19 | 0 | -369 | -369 | -723 | -659 | -570 | -589 | -628 | -543 | -638 | -577 | -378 | 45793 |
| 10 | 20 | 0 | -342 | -372 | -693 | -684 | -561 | -546 | -525 | -394 | -354 | -70 | 806 | 47358 |
| 10 | 21 | 0 | -357 | -345 | -690 | -623 | -571 | -546 | -562 | -458 | -440 | -272 | 302 | 47105 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. aerogenes* FRHEAE2 with EHC-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -338 | -338 | -653 | -595 | -494 | -503 | -564 | -491 | -555 | -467 | -226 | 46647 |
| 10 | 23 | 0 | -333 | -339 | -653 | -623 | -562 | -522 | -565 | -461 | -431 | -302 | 137 | 46732 |
| 10 | 24 | 0 | -327 | -345 | -653 | -617 | -571 | -525 | -565 | -440 | -421 | -257 | 271 | 46943 |
| 10 | 25 | 0 | -354 | -378 | -680 | -644 | -552 | -552 | -558 | -430 | -433 | -155 | 666 | 47300 |
| 10 | 26 | 0 | -381 | -387 | -699 | -650 | -552 | -546 | -537 | -406 | -384 | -27 | 1178 | 47425 |
| 10 | 27 | 0 | -336 | -361 | -687 | -620 | -525 | -541 | -492 | -370 | -156 | 714 | 2795 | 47153 |
| 10 | 28 | 0 | -342 | -372 | -699 | -650 | -574 | -543 | -546 | -406 | -381 | 34 | 1624 | 47245 |
| 10 | 29 | 0 | -363 | -345 | -669 | -608 | -562 | -547 | -540 | -388 | -370 | -189 | 909 | 46891 |
| 10 | 30 | 0 | -314 | -336 | -632 | -601 | -528 | -519 | -531 | -421 | -403 | -262 | 238 | 48014 |
| 10 | 31 | 0 | -324 | -336 | -666 | -611 | -550 | -501 | -519 | -388 | -345 | -40 | 665 | 46497 |
| 10 | 32 | 0 | -333 | -357 | -647 | -620 | -565 | -522 | -559 | -415 | -382 | -174 | 436 | 47300 |
| 5 | 1 | 0 | -357 | -375 | -675 | -635 | -534 | -546 | -513 | -421 | -379 | -140 | 421 | 46830 |
| 5 | 2 | 0 | -363 | -378 | -693 | -644 | -570 | -574 | -610 | -531 | -650 | -574 | -412 | 46909 |
| 5 | 3 | 0 | -360 | -387 | -699 | -659 | -561 | -564 | -610 | -552 | -696 | -638 | -549 | -326 |
| 5 | 4 | 0 | -351 | -360 | -678 | -644 | -559 | -553 | -565 | -427 | -458 | -247 | 354 | 47114 |
| 5 | 5 | 0 | -342 | -321 | -666 | -617 | -553 | -525 | -565 | -486 | -553 | -422 | -95 | 46567 |
| 5 | 6 | 0 | -342 | -348 | -675 | -611 | -553 | -516 | -553 | -467 | -464 | -361 | 45 | 46619 |
| 5 | 7 | 0 | -320 | -329 | -644 | -631 | -531 | -521 | -549 | -494 | -555 | -485 | -216 | 46162 |
| 5 | 8 | 0 | -321 | -345 | -635 | -592 | -559 | -537 | -589 | -498 | -635 | -574 | -373 | 46399 |
| 5 | 9 | 0 | -363 | -396 | -711 | -656 | -583 | -583 | -604 | -555 | -695 | -668 | -576 | -375 |
| 5 | 10 | 0 | -360 | -381 | -699 | -653 | -555 | -552 | -595 | -509 | -540 | -381 | -106 | 47148 |
| 5 | 11 | 0 | -348 | -385 | -681 | -629 | -558 | -528 | -562 | -482 | -464 | -290 | 61 | 47260 |
| 5 | 12 | 0 | -330 | -360 | -681 | -644 | -528 | -546 | -549 | -418 | -436 | -165 | 971 | 47285 |
| 5 | 13 | 0 | -318 | -345 | -663 | -623 | -550 | -534 | -583 | -516 | -656 | -568 | -373 | 46643 |
| 5 | 14 | 0 | -333 | -354 | -681 | -614 | -559 | -544 | -571 | -522 | -571 | -489 | -244 | 46457 |
| 5 | 15 | 0 | -312 | -330 | -666 | -620 | -559 | -537 | -586 | -528 | -641 | -611 | -504 | -330 |
| 5 | 16 | 0 | -336 | -342 | -623 | -601 | -583 | -540 | -543 | -473 | -479 | -281 | 299 | 47001 |
| 5 | 17 | 0 | -363 | -375 | -699 | -635 | -564 | -570 | -586 | -525 | -607 | -494 | -223 | 46980 |
| 5 | 18 | 0 | -366 | -372 | -689 | -659 | -567 | -576 | -589 | -531 | -604 | -464 | -232 | 47212 |
| 5 | 19 | 0 | -342 | -361 | -675 | -626 | -562 | -547 | -577 | -522 | -657 | -623 | -541 | -327 |
| 5 | 20 | 0 | -336 | -360 | -656 | -635 | -534 | -519 | -522 | -388 | -372 | 55 | 1263 | 47446 |
| 5 | 21 | 0 | -351 | -373 | -672 | -629 | -540 | -510 | -562 | -458 | -534 | -351 | 12 | 46845 |
| 5 | 22 | 0 | -336 | -348 | -632 | -598 | -546 | -516 | -555 | -482 | -528 | -436 | -131 | 46305 |
| 5 | 23 | 0 | -305 | -338 | -641 | -613 | -534 | -488 | -488 | -360 | -311 | 16 | 1072 | 46671 |
| 5 | 24 | 0 | -336 | -351 | -650 | -619 | -561 | -546 | -583 | -519 | -580 | -488 | -214 | 46903 |
| 5 | 25 | 0 | -387 | -393 | -720 | -674 | -561 | -564 | -558 | -421 | -345 | -58 | 855 | 47596 |
| 5 | 26 | 0 | -348 | -378 | -686 | -632 | -555 | -561 | -583 | -506 | -537 | -375 | 95 | 47917 |
| 5 | 27 | 0 | -376 | -409 | -705 | -675 | -577 | -583 | -611 | -537 | -657 | -565 | -367 | 47403 |
| 5 | 28 | 0 | -360 | -363 | -702 | -650 | -549 | -552 | -570 | -497 | -586 | -442 | -170 | 47291 |
| 5 | 29 | 0 | -345 | -357 | -674 | -632 | -558 | -561 | -577 | -519 | -641 | -558 | -372 | 47010 |
| 5 | 30 | 0 | -360 | -360 | -659 | -644 | -570 | -543 | -580 | -488 | -525 | -412 | -55 | 47352 |
| 5 | 31 | 0 | -342 | -348 | -653 | -614 | -546 | -528 | -522 | -394 | -321 | 43 | 1212 | 47257 |
| 5 | 32 | 0 | -342 | -357 | -635 | -617 | -568 | -534 | -531 | -437 | -379 | -174 | 592 | 47693 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*C. freundii* NCTC 9750 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -296 | -342 | -397 | -397 | -409 | -324 | -354 | -391 | -425 | -486 | -473 | -296 |
| 20 | 2 | 0 | -293 | -360 | -397 | -397 | -397 | -348 | -354 | -379 | -412 | -476 | -486 | -296 |
| 20 | 3 | 0 | -336 | -394 | -433 | -433 | -445 | -369 | -412 | -409 | -436 | -506 | -516 | -299 |
| 20 | 4 | 0 | -305 | -382 | -400 | -418 | -437 | -345 | -348 | -333 | -278 | -40 | 561 | 34872 |
| 20 | 5 | 0 | -314 | -390 | -427 | -424 | -439 | -381 | -397 | -430 | -458 | -516 | -522 | -342 |
| 20 | 6 | 0 | -326 | -415 | -436 | -445 | -464 | -372 | -384 | -412 | -439 | -510 | -516 | -311 |
| 20 | 7 | 0 | -330 | -397 | -403 | -430 | -470 | -369 | -406 | -421 | -430 | -504 | -494 | -323 |
| 20 | 8 | 0 | -336 | -413 | -440 | -437 | -483 | -394 | -413 | -419 | -477 | -535 | -519 | -336 |
| 20 | 9 | 0 | -333 | -381 | -415 | -421 | -439 | -375 | -388 | -397 | -439 | -497 | -494 | 32769 |
| 20 | 10 | 0 | -290 | -348 | -390 | -405 | -424 | -329 | -357 | -390 | -393 | -467 | -463 | -296 |
| 20 | 11 | 0 | -321 | -397 | -406 | -422 | -437 | -373 | -370 | -397 | -443 | -498 | -501 | -327 |
| 20 | 12 | 0 | -327 | -370 | -403 | -431 | -431 | -342 | -367 | -391 | -415 | -492 | -489 | -306 |
| 20 | 13 | 0 | -317 | -400 | -440 | -430 | -446 | -366 | -385 | -433 | -443 | -522 | -507 | -336 |
| 20 | 14 | 0 | -327 | -391 | -418 | -446 | -449 | -382 | -391 | -406 | -446 | -504 | -519 | -333 |
| 20 | 15 | 0 | -315 | -403 | -406 | -421 | -437 | -357 | -397 | -415 | -427 | -495 | -504 | -315 |
| 20 | 16 | 0 | -326 | -412 | -424 | -442 | -451 | -372 | -412 | -424 | -467 | -506 | -513 | -354 |
| 20 | 17 | 0 | -311 | -385 | -406 | -415 | -430 | -348 | -385 | -394 | -433 | -476 | -510 | -290 |
| 20 | 18 | 0 | -318 | -382 | -406 | -418 | -418 | -339 | -360 | -339 | -308 | -177 | 262 | 33673 |
| 20 | 19 | 0 | -318 | -385 | -421 | -430 | -434 | -366 | -397 | -409 | -446 | -498 | -507 | -311 |
| 20 | 20 | 0 | -342 | -387 | -427 | -433 | -458 | -375 | -397 | -415 | -473 | -522 | -528 | -308 |
| 20 | 21 | 0 | -326 | -400 | -424 | -445 | -464 | -381 | -415 | -433 | -448 | -513 | -516 | 31610 |
| 20 | 22 | 0 | -372 | -397 | -445 | -451 | -464 | -387 | -393 | -372 | -323 | -335 | -152 | 31814 |
| 20 | 23 | 0 | -354 | -427 | -445 | -473 | -467 | -406 | -427 | -439 | -470 | -537 | -540 | -354 |
| 20 | 24 | 0 | -336 | -406 | -430 | -439 | -455 | -375 | -381 | -421 | -451 | -513 | -506 | -336 |
| 20 | 25 | 0 | -312 | -363 | -397 | -424 | -431 | -348 | -400 | -400 | -421 | -476 | -485 | -318 |
| 20 | 26 | 0 | -315 | -373 | -382 | -403 | -415 | -345 | -373 | -388 | -434 | -467 | -483 | -275 |
| 20 | 27 | 0 | -299 | -373 | -406 | -425 | -425 | -367 | -354 | -327 | -278 | -177 | 24 | 31966 |
| 20 | 28 | 0 | -342 | -397 | -427 | -436 | -446 | -363 | -388 | -421 | -442 | -507 | -528 | 17702 |
| 20 | 29 | 0 | -308 | -382 | -443 | -434 | -449 | -363 | -379 | -427 | -427 | -504 | -504 | -311 |
| 20 | 30 | 0 | -348 | -409 | -461 | -467 | -467 | -403 | -412 | -424 | -455 | -531 | -525 | -329 |
| 20 | 31 | 0 | -327 | -397 | -403 | -427 | -446 | -363 | -403 | -406 | -434 | -504 | -498 | -339 |
| 20 | 32 | 0 | -341 | -421 | -442 | -451 | -460 | -378 | -421 | -424 | -457 | -521 | -515 | -354 |
| 10 | 1 | 0 | -318 | -397 | -421 | -431 | -437 | -354 | -403 | -415 | -437 | -495 | -507 | -305 |
| 10 | 2 | 0 | -327 | -373 | -397 | -419 | -431 | -333 | -364 | -379 | -373 | -330 | -150 | 33001 |
| 10 | 3 | 0 | -332 | -378 | -421 | -421 | -430 | -338 | -396 | -390 | -433 | -494 | -473 | -314 |
| 10 | 4 | 0 | -363 | -421 | -439 | -445 | -467 | -375 | -418 | -439 | -464 | -516 | -522 | -336 |
| 10 | 5 | 0 | -345 | -406 | -430 | -443 | -464 | -397 | -415 | -406 | -452 | -522 | -528 | -345 |
| 10 | 6 | 0 | -345 | -391 | -437 | -440 | -455 | -376 | -400 | -406 | -446 | -510 | -507 | -351 |
| 10 | 7 | 0 | -330 | -397 | -397 | -449 | -470 | -376 | -397 | -397 | -440 | -510 | -498 | -345 |
| 10 | 8 | 0 | -348 | -427 | -461 | -467 | -482 | -394 | -406 | -415 | -451 | -534 | -519 | -354 |
| 10 | 9 | 0 | -329 | -400 | -424 | -451 | -454 | -387 | -415 | -412 | -409 | -366 | -216 | 33929 |
| 10 | 10 | 0 | -357 | -403 | -433 | -470 | -476 | -406 | -430 | -439 | -445 | -531 | -522 | -329 |
| 10 | 11 | 0 | -363 | -427 | -445 | -464 | -458 | -403 | -409 | -433 | -467 | -558 | -534 | -366 |
| 10 | 12 | 0 | -369 | -439 | -451 | -463 | -470 | -405 | -433 | -457 | -473 | -537 | -531 | -351 |
| 10 | 13 | 0 | -373 | -449 | -473 | -477 | -486 | -419 | -434 | -409 | -388 | -312 | -77 | 32403 |
| 10 | 14 | 0 | -364 | -434 | -464 | -470 | -495 | -403 | -419 | -440 | -470 | -550 | -544 | -373 |
| 10 | 15 | 0 | -364 | -443 | -483 | -483 | -504 | -422 | -434 | -464 | -477 | -574 | -538 | -379 |
| 10 | 16 | 0 | -367 | -458 | -467 | -483 | -501 | -403 | -443 | -452 | -489 | -556 | -550 | -373 |
| 10 | 17 | 0 | -360 | -399 | -448 | -464 | -454 | -396 | -421 | -427 | -476 | -506 | -531 | -338 |
| 10 | 18 | 0 | -345 | -415 | -427 | -448 | -458 | -387 | -409 | -424 | -448 | -516 | -522 | 27404 |
| 10 | 19 | 0 | -378 | -427 | -458 | -464 | -476 | -406 | -421 | -439 | -461 | -534 | -528 | -351 |
| 10 | 20 | 0 | -367 | -431 | -464 | -467 | -489 | -416 | -440 | -446 | -470 | -535 | -541 | -354 |
| 10 | 21 | 0 | -351 | -418 | -448 | -464 | -491 | -397 | -427 | -451 | -470 | -531 | -543 | -369 |

**Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.**

*C. freundii* NCTC 9750 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -345 | -428 | -455 | -474 | -458 | -403 | -440 | -458 | -474 | -538 | -544 | -376 |
| 10 | 23 | 0 | -361 | -440 | -477 | -470 | -498 | -416 | -440 | -431 | -464 | -538 | -541 | -379 |
| 10 | 24 | 0 | -369 | -455 | -479 | -488 | -510 | -421 | -443 | -461 | -461 | -452 | -235 | 31420 |
| 10 | 25 | 0 | -333 | -403 | -439 | -436 | -445 | -381 | -415 | -421 | -464 | -500 | -506 | -326 |
| 10 | 26 | 0 | -345 | -391 | -439 | -433 | -430 | -378 | -403 | -424 | -452 | -494 | -528 | -336 |
| 10 | 27 | 0 | -348 | -421 | -448 | -458 | -467 | -387 | -403 | -415 | -464 | -528 | -519 | -348 |
| 10 | 28 | 0 | -370 | -428 | -473 | -483 | -492 | -400 | -437 | -446 | -477 | -544 | -544 | -379 |
| 10 | 29 | 0 | -357 | -424 | -467 | -482 | -494 | -403 | -433 | -454 | -470 | -537 | -540 | -369 |
| 10 | 30 | 0 | -363 | -439 | -470 | -473 | -500 | -415 | -439 | -448 | -479 | -543 | -543 | -378 |
| 10 | 31 | 0 | -375 | -412 | -458 | -436 | -448 | -363 | -403 | -406 | -442 | -519 | -485 | -360 |
| 10 | 32 | 0 | -357 | -442 | -473 | -470 | -479 | -399 | -424 | -442 | -470 | -537 | -528 | -357 |
| 5 | 1 | 0 | -330 | -412 | -439 | -442 | -452 | -363 | -433 | -430 | -445 | -500 | -510 | -342 |
| 5 | 2 | 0 | -351 | -409 | -451 | -454 | -454 | -384 | -433 | -424 | -464 | -534 | -531 | -351 |
| 5 | 3 | 0 | -354 | -421 | -446 | -449 | -473 | -403 | -403 | -442 | -467 | -510 | -516 | -333 |
| 5 | 4 | 0 | -376 | -437 | -498 | -470 | -492 | -422 | -431 | -455 | -486 | -544 | -538 | -385 |
| 5 | 5 | 0 | -357 | -424 | -454 | -473 | -485 | -412 | -433 | -454 | -454 | -531 | -537 | -375 |
| 5 | 6 | 0 | -345 | -421 | -473 | -482 | -482 | -400 | -424 | -430 | -457 | -540 | -543 | -351 |
| 5 | 7 | 0 | -354 | -442 | -470 | -476 | -488 | -412 | -442 | -442 | -470 | -540 | -537 | -375 |
| 5 | 8 | 0 | -364 | -458 | -476 | -495 | -495 | -431 | -428 | -464 | -486 | -541 | -538 | -388 |
| 5 | 9 | 0 | -366 | -424 | -454 | -451 | -463 | -396 | -436 | -424 | -460 | -524 | -509 | -360 |
| 5 | 10 | 0 | -351 | -400 | -436 | -427 | -467 | -381 | -409 | -424 | -436 | -497 | -522 | -348 |
| 5 | 11 | 0 | -363 | -424 | -445 | -464 | -470 | -384 | -418 | -436 | -473 | -528 | -528 | -329 |
| 5 | 12 | 0 | -342 | -418 | -457 | -445 | -479 | -390 | -409 | -445 | -464 | -534 | -522 | -357 |
| 5 | 13 | 0 | -364 | -434 | -480 | -470 | -489 | -382 | -419 | -452 | -458 | -525 | -525 | -364 |
| 5 | 14 | 0 | -344 | -451 | -470 | -470 | -494 | -412 | -442 | -448 | -467 | -537 | -543 | -384 |
| 5 | 15 | 0 | -357 | -430 | -460 | -448 | -494 | -402 | -427 | -436 | -476 | -540 | -546 | -372 |
| 5 | 16 | 0 | -351 | -439 | -476 | -470 | -494 | -406 | -424 | -448 | -479 | -555 | -531 | -375 |
| 5 | 17 | 0 | -369 | -430 | -442 | -439 | -445 | -375 | -433 | -424 | -454 | -519 | -525 | -342 |
| 5 | 18 | 0 | -357 | -412 | -458 | -452 | -467 | -382 | -397 | -440 | -452 | -513 | -525 | -348 |
| 5 | 19 | 0 | -335 | -397 | -430 | -454 | -458 | -384 | -412 | -415 | -436 | -494 | -494 | -332 |
| 5 | 20 | 0 | -369 | -446 | -461 | -479 | -498 | -400 | -434 | -458 | -482 | -559 | -546 | -394 |
| 5 | 21 | 0 | -348 | -421 | -463 | -463 | -476 | -390 | -418 | -436 | -467 | -518 | -528 | -357 |
| 5 | 22 | 0 | -347 | -433 | -454 | -460 | -497 | -387 | -439 | -436 | -466 | -549 | -537 | -351 |
| 5 | 23 | 0 | -375 | -464 | -485 | -482 | -501 | -418 | -455 | -476 | -494 | -592 | -562 | -378 |
| 5 | 24 | 0 | -369 | -458 | -491 | -485 | -501 | -430 | -437 | -464 | -504 | -540 | -562 | -379 |
| 5 | 25 | 0 | -326 | -384 | -427 | -430 | -455 | -375 | -412 | -436 | -455 | -488 | -510 | -317 |
| 5 | 26 | 0 | -351 | -418 | -448 | -463 | -470 | -384 | -412 | -418 | -454 | -509 | -518 | -344 |
| 5 | 27 | 0 | -351 | -424 | -454 | -439 | -476 | -402 | -418 | -433 | -445 | -515 | -537 | -357 |
| 5 | 28 | 0 | -366 | -430 | -479 | -467 | -498 | -415 | -430 | -464 | -482 | -549 | -549 | -369 |
| 5 | 29 | 0 | -369 | -439 | -473 | -506 | -500 | -415 | -439 | -473 | -482 | -561 | -552 | -363 |
| 5 | 30 | 0 | -363 | -446 | -488 | -494 | -516 | -427 | -433 | -455 | -476 | -574 | -534 | 31936 |
| 5 | 31 | 0 | -360 | -458 | -494 | -510 | -528 | -424 | -443 | -488 | -497 | -568 | -565 | -369 |
| 5 | 32 | 0 | -390 | -476 | -512 | -509 | -528 | -433 | -439 | -485 | -522 | -567 | -583 | -406 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*K. pneumoniae* NCTC 10896 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -31 | -52 | -80 | -12 | 24 | 250 | 549 | 1965 | 2527 | 4923 | 8219 | 36276 |
| 20 | 2 | 0 | 49 | 61 | 192 | 592 | 1282 | 3360 | 6696 | 14628 | 16160 | 21120 | 23836 | 36111 |
| 20 | 3 | 0 | 95 | 180 | 434 | 1215 | 2360 | 5491 | 9468 | 17421 | 18154 | 21502 | 23360 | 35233 |
| 20 | 4 | 0 | 6 | -28 | -34 | 6 | 97 | 470 | 1053 | 3131 | 3836 | 6610 | 10962 | 34158 |
| 20 | 5 | 0 | -13 | -31 | -65 | -25 | -31 | 109 | 213 | 689 | 854 | 1663 | 2817 | 33843 |
| 20 | 6 | 0 | 6 | -43 | -71 | -37 | -22 | 131 | 241 | 732 | 802 | 1596 | 2762 | 33242 |
| 20 | 7 | 0 | -9 | -54 | -64 | -61 | -64 | 55 | 107 | 355 | 309 | 739 | 1423 | 32001 |
| 20 | 8 | 0 | 13 | -33 | -15 | 25 | 80 | 290 | 446 | 947 | 980 | 1685 | 2787 | 26104 |
| 20 | 9 | 0 | -12 | -58 | 15 | 216 | 677 | 1501 | 2838 | 5853 | 6470 | 9470 | 12684 | 36343 |
| 20 | 10 | 0 | -12 | -42 | -45 | -49 | -58 | 40 | 74 | 357 | 403 | 977 | 2717 | 33868 |
| 20 | 11 | 0 | 18 | 3 | 21 | 107 | 263 | 833 | 1599 | 3424 | 3888 | 6104 | 7948 | 32672 |
| 20 | 12 | 0 | -31 | -43 | -65 | -68 | -77 | 36 | 64 | 347 | 405 | 994 | 1992 | 33364 |
| 20 | 13 | 0 | 19 | 10 | 31 | 162 | 431 | 1304 | 2811 | 7682 | 9587 | 15166 | 19967 | 33728 |
| 20 | 14 | 0 | 28 | 6 | 34 | 186 | 519 | 1514 | 3327 | 8369 | 9779 | 14821 | 19252 | 33328 |
| 20 | 15 | 0 | -7 | -62 | -68 | -77 | -107 | -34 | -62 | -25 | -156 | -150 | -107 | 33 |
| 20 | 16 | 0 | 55 | 55 | 174 | 500 | 946 | 2130 | 3543 | 6818 | 7660 | 11732 | 16362 | 32815 |
| 20 | 17 | 0 | -25 | -71 | -74 | -71 | -95 | 0 | -74 | -43 | -162 | -153 | -116 | 55 |
| 20 | 18 | 0 | -28 | -71 | -92 | -77 | -113 | -34 | -80 | -52 | -162 | -183 | -119 | 222 |
| 20 | 19 | 0 | -24 | -79 | -73 | -48 | -52 | 58 | 98 | 440 | 492 | 1224 | 2445 | 32864 |
| 20 | 20 | 0 | 22 | 19 | 77 | 327 | 654 | 1877 | 3867 | 9074 | 10594 | 15590 | 21178 | 34464 |
| 20 | 21 | 0 | 6 | 15 | 79 | 323 | 717 | 1916 | 3769 | 8536 | 9928 | 14961 | 19856 | 33724 |
| 20 | 22 | 0 | -12 | -39 | -54 | -33 | -18 | 193 | 470 | 1389 | 1707 | 3376 | 5503 | 32541 |
| 20 | 23 | 0 | 7 | 13 | 40 | 190 | 397 | 1279 | 2604 | 6257 | 7460 | 11397 | 15886 | 32672 |
| 20 | 24 | 0 | 22 | -3 | 34 | 129 | 300 | 943 | 1935 | 4945 | 5940 | 9712 | 14281 | 33456 |
| 20 | 25 | 0 | -49 | -83 | -95 | -80 | -86 | 18 | 79 | 412 | 500 | 1230 | 2435 | 35967 |
| 20 | 26 | 0 | -15 | -73 | -76 | -30 | -33 | 147 | 296 | 998 | 1258 | 2671 | 4691 | 33691 |
| 20 | 27 | 0 | -31 | -80 | -86 | -76 | -70 | 55 | 137 | 598 | 729 | 1688 | 3159 | 33093 |
| 20 | 28 | 0 | -40 | -40 | -55 | -61 | -101 | -27 | -52 | -24 | -140 | -137 | -119 | 61 |
| 20 | 29 | 0 | 12 | 9 | 24 | 189 | 433 | 1276 | 2710 | 6986 | 8402 | 13288 | 18666 | 33603 |
| 20 | 30 | 0 | 0 | -18 | 43 | 366 | 952 | 2906 | 5707 | 12507 | 14186 | 20861 | 25524 | 35239 |
| 20 | 31 | 0 | -12 | -58 | -91 | -85 | -109 | -45 | -94 | -36 | -161 | -170 | -122 | 10 |
| 20 | 32 | 0 | -12 | -48 | -67 | -79 | -106 | -27 | -58 | -36 | -155 | -152 | -116 | 13 |
| 10 | 1 | 0 | -46 | -82 | -107 | -92 | -76 | 58 | 177 | 726 | 897 | 1917 | 3198 | 36136 |
| 10 | 2 | 0 | 12 | -3 | 61 | 323 | 714 | 1974 | 3900 | 8847 | 10343 | 15403 | 19993 | 33804 |
| 10 | 3 | 0 | -30 | -51 | -97 | -79 | -109 | -30 | -76 | -42 | -180 | -164 | -140 | 31 |
| 10 | 4 | 0 | -49 | -58 | -86 | -58 | -65 | 64 | 167 | 674 | 818 | 1733 | 3009 | 31692 |
| 10 | 5 | 0 | -12 | -61 | -46 | -52 | -85 | 3 | -15 | 21 | -73 | 28 | 195 | 31533 |
| 10 | 6 | 0 | -21 | -42 | -73 | -73 | -85 | -12 | -49 | -30 | -140 | -128 | -106 | 37 |
| 10 | 7 | 0 | -21 | -39 | -67 | -85 | -82 | -24 | -55 | -33 | -146 | -180 | -125 | 25 |
| 10 | 8 | 0 | -16 | -58 | -34 | -13 | 55 | 436 | 1004 | 3143 | 4227 | 7727 | 12058 | 33993 |
| 10 | 9 | 0 | -40 | -46 | -77 | -58 | -43 | 107 | 247 | 927 | 1086 | 2432 | 4349 | 36047 |
| 10 | 10 | 0 | -25 | -74 | -98 | -71 | -61 | 30 | 88 | 451 | 528 | 1135 | 2383 | 33138 |
| 10 | 11 | 0 | -42 | -76 | -106 | -73 | -106 | -12 | -18 | 113 | 43 | 296 | 797 | 32404 |
| 10 | 12 | 0 | -36 | -67 | -91 | -79 | -106 | -30 | -73 | -39 | -146 | -137 | -137 | 37 |
| 10 | 13 | 0 | -3 | -33 | -12 | 86 | 183 | 696 | 1563 | 4105 | 4883 | 7615 | 10102 | 31134 |
| 10 | 14 | 0 | -18 | -40 | -61 | -67 | -79 | -15 | -55 | -15 | -140 | -140 | -91 | 61 |
| 10 | 15 | 0 | -12 | -49 | -67 | -55 | -76 | 25 | 46 | 293 | 287 | 800 | 1804 | 32245 |
| 10 | 16 | 0 | -3 | -46 | 0 | 100 | 229 | 912 | 2194 | 6507 | 8316 | 13731 | 19020 | 34554 |
| 10 | 17 | 0 | -27 | -64 | -85 | -79 | -100 | 13 | 31 | 251 | 226 | 696 | 1227 | 35669 |
| 10 | 18 | 0 | -22 | -37 | 33 | 275 | 598 | 1681 | 3925 | 10496 | 12641 | 18159 | 22695 | 34530 |
| 10 | 19 | 0 | -12 | -15 | 34 | 260 | 473 | 1285 | 2317 | 4618 | 5195 | 7624 | 10447 | 32461 |
| 10 | 20 | 0 | -52 | -89 | -116 | -95 | -119 | -58 | -77 | -61 | -177 | -184 | -135 | 15 |
| 10 | 21 | 0 | -21 | -46 | -70 | -52 | -58 | 43 | 101 | 369 | 449 | 1065 | 2198 | 31890 |

**Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.**

***K. pneumoniae* NCTC 10896 with 4-MU-GAL**

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | 3 | -30 | -49 | -21 | -49 | 64 | 162 | 617 | 757 | 1657 | 2893 | 31488 |
| 10 | 23 | 0 | -18 | -39 | -45 | 0 | 19 | 303 | 702 | 2479 | 3269 | 6510 | 10557 | 32138 |
| 10 | 24 | 0 | -9 | -49 | -55 | -55 | -79 | -3 | -46 | -27 | -134 | -161 | -107 | 22 |
| 10 | 25 | 0 | -34 | -98 | -95 | -61 | -46 | 103 | 250 | 842 | 992 | 2209 | 3503 | 35678 |
| 10 | 26 | 0 | -43 | -92 | -95 | -71 | -58 | 58 | 134 | 521 | 644 | 1318 | 2749 | 32729 |
| 10 | 27 | 0 | -39 | -73 | -73 | -36 | -52 | 122 | 220 | 794 | 1004 | 2183 | 4020 | 32181 |
| 10 | 28 | 0 | -21 | -61 | -100 | -82 | -104 | -27 | -58 | -33 | -155 | -168 | -113 | 34 |
| 10 | 29 | 0 | -3 | -43 | -37 | 31 | 104 | 537 | 1276 | 3717 | 4548 | 8158 | 11897 | 32013 |
| 10 | 30 | 0 | -21 | -37 | -40 | 9 | 82 | 415 | 992 | 3299 | 4471 | 8955 | 14158 | 32675 |
| 10 | 31 | 0 | -15 | -43 | -67 | -43 | -64 | 43 | 58 | 327 | 348 | 888 | 1926 | 32049 |
| 10 | 32 | 0 | -12 | -67 | -104 | -101 | -113 | -46 | -95 | -49 | -165 | -174 | -144 | -28 |
| 5 | 1 | 0 | -64 | -88 | -134 | -104 | -116 | -6 | 28 | 315 | 336 | 952 | 1905 | 35382 |
| 5 | 2 | 0 | -55 | -119 | -131 | -113 | -125 | -64 | -101 | -64 | -168 | -195 | -144 | 30 |
| 5 | 3 | 0 | -46 | -58 | -22 | 76 | 268 | 830 | 1553 | 3134 | 3506 | 5279 | 7453 | 31279 |
| 5 | 4 | 0 | -40 | -77 | -95 | -80 | -113 | 15 | 36 | 323 | 338 | 903 | 2002 | 32192 |
| 5 | 5 | 0 | -33 | -94 | -109 | -85 | -91 | 25 | 43 | 260 | 284 | 794 | 1853 | 32022 |
| 5 | 6 | 0 | -37 | -65 | -101 | -62 | -92 | 21 | 112 | 460 | 515 | 1211 | 2151 | 32094 |
| 5 | 7 | 0 | -43 | -104 | -98 | -83 | -126 | -49 | -58 | -55 | -171 | -187 | -147 | 0 |
| 5 | 8 | 0 | -31 | -92 | -104 | -86 | -101 | 27 | 76 | 372 | 430 | 1156 | 2447 | 34445 |
| 5 | 9 | 0 | -52 | -101 | -110 | -82 | -92 | 18 | 55 | 415 | 482 | 1260 | 2344 | 35238 |
| 5 | 10 | 0 | -64 | -101 | -134 | -122 | -149 | -85 | -116 | -85 | -192 | -192 | -162 | -15 |
| 5 | 11 | 0 | -40 | -83 | -101 | -107 | -122 | -43 | -89 | -71 | -147 | -177 | -144 | 12 |
| 5 | 12 | 0 | -43 | -101 | -135 | -101 | -119 | -58 | -61 | 30 | -58 | 55 | 369 | 32085 |
| 5 | 13 | 0 | -24 | -85 | -109 | -85 | -109 | -36 | -67 | -45 | -167 | -171 | -119 | 52 |
| 5 | 14 | 0 | -31 | -31 | -34 | 112 | 274 | 952 | 2063 | 4910 | 5884 | 9864 | 14625 | 32299 |
| 5 | 15 | 0 | -49 | -98 | -110 | -104 | -122 | -46 | -95 | -49 | -186 | -208 | -174 | -19 |
| 5 | 16 | 0 | -76 | -119 | -140 | -140 | -146 | -70 | -119 | -82 | -226 | -217 | -165 | -12 |
| 5 | 17 | 0 | -46 | -86 | -101 | -95 | -92 | 24 | 113 | 540 | 659 | 1642 | 2915 | 35180 |
| 5 | 18 | 0 | -73 | -109 | -143 | -125 | -155 | -91 | -122 | -100 | -195 | -232 | -170 | -27 |
| 5 | 19 | 0 | -31 | -86 | -107 | -89 | -141 | -61 | -80 | -80 | -168 | -193 | -141 | -16 |
| 5 | 20 | 0 | -40 | -77 | -113 | -92 | -132 | -68 | -74 | -55 | -168 | -171 | -119 | 58 |
| 5 | 21 | 0 | -28 | -89 | -107 | -74 | -77 | 61 | 76 | 375 | 421 | 1049 | 2304 | 32622 |
| 5 | 22 | 0 | -52 | -89 | -98 | -92 | -122 | -58 | -83 | -46 | -171 | -193 | -177 | -3 |
| 5 | 23 | 0 | -40 | -92 | -107 | -95 | -132 | -37 | -55 | -13 | -125 | -31 | 107 | 32772 |
| 5 | 24 | 0 | -40 | -107 | -107 | -113 | -122 | -40 | -92 | -64 | -186 | -229 | 171 | 34533 |
| 5 | 25 | 0 | -49 | -83 | -128 | -122 | -125 | -64 | -98 | -73 | -192 | -223 | -153 | 0 |
| 5 | 26 | 0 | -34 | -82 | -104 | -131 | -128 | -46 | -98 | -58 | -183 | -208 | -131 | -9 |
| 5 | 27 | 0 | -43 | -79 | -122 | -116 | -140 | -67 | -82 | -79 | -189 | -198 | -162 | -12 |
| 5 | 28 | 0 | -46 | -76 | -110 | -85 | -113 | 3 | 58 | 333 | 421 | 1102 | 2060 | 35690 |
| 5 | 29 | 0 | -34 | -67 | -125 | -89 | -116 | -37 | -83 | -28 | -128 | -83 | -16 | 34939 |
| 5 | 30 | 0 | -43 | -80 | -116 | -83 | -119 | -61 | -83 | -64 | -177 | -195 | -205 | -18 |
| 5 | 31 | 0 | -28 | -89 | -80 | -98 | -122 | -55 | -80 | -64 | -177 | -190 | -129 | 34426 |
| 5 | 32 | 0 | -52 | -85 | -104 | -79 | -34 | 113 | 275 | 977 | 1334 | 3000 | 4990 | 35672 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. cloacae* NCTC 11936 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -15 | -55 | -76 | -85 | -46 | -27 | 214 | 235 | 315 | 708 | 1380 | 34360 |
| 20 | 2 | 0 | -24 | -30 | -67 | -88 | -82 | -55 | 83 | -70 | -106 | -177 | -131 | 83 |
| 20 | 3 | 0 | -33 | -39 | -79 | -9 | 190 | 773 | 2448 | 4502 | 6040 | 9050 | 12251 | 35321 |
| 20 | 4 | 0 | -27 | -48 | -97 | -106 | -100 | -79 | 74 | -94 | -106 | -180 | -131 | 86 |
| 20 | 5 | 0 | -3 | -34 | -64 | -101 | -76 | -49 | 113 | -49 | -61 | -159 | -107 | 107 |
| 20 | 6 | 0 | 3 | -3 | -64 | -80 | -52 | 6 | 369 | 1031 | 2087 | 6375 | 12373 | 36853 |
| 20 | 7 | 0 | -30 | -30 | -58 | -55 | 89 | 718 | 3422 | 8729 | 12959 | 18630 | 22085 | 36597 |
| 20 | 8 | 0 | -51 | -42 | -91 | -67 | 31 | 611 | 2772 | 8079 | 12004 | 17769 | 21352 | 36054 |
| 20 | 9 | 0 | -18 | -33 | -76 | -106 | -67 | -82 | 89 | -61 | -110 | -177 | -131 | 80 |
| 20 | 10 | 0 | -27 | -64 | -82 | -103 | -76 | -73 | 89 | -67 | -113 | -143 | -161 | 61 |
| 20 | 11 | 0 | -18 | -18 | -58 | -83 | -70 | -64 | 79 | -55 | -92 | -131 | -89 | 113 |
| 20 | 12 | 0 | -27 | -24 | -46 | -70 | -30 | 125 | 1026 | 3058 | 5466 | 11729 | 17464 | 35947 |
| 20 | 13 | 0 | -27 | 15 | 3 | 223 | 729 | 2161 | 6034 | 10420 | 13127 | 17693 | 21224 | 36072 |
| 20 | 14 | 0 | 3 | -13 | -37 | -22 | 122 | 650 | 3378 | 9894 | 14674 | 20167 | 23235 | 36065 |
| 20 | 15 | 0 | -40 | -36 | -58 | -110 | -88 | -70 | 76 | -82 | -97 | -177 | -146 | 43 |
| 20 | 16 | 0 | -58 | -73 | -125 | -147 | -125 | -107 | 73 | -113 | -122 | -214 | -162 | 46 |
| 20 | 17 | 0 | -18 | -27 | -46 | 9 | 241 | 885 | 3550 | 7893 | 11189 | 17064 | 21123 | 37100 |
| 20 | 18 | 0 | -18 | -24 | -3 | 214 | 903 | 2817 | 8668 | 14988 | 18404 | 22966 | 25362 | 36102 |
| 20 | 19 | 0 | -27 | -3 | -27 | 98 | 397 | 1087 | 2652 | 4071 | 5018 | 6733 | 8616 | 33029 |
| 20 | 20 | 0 | -30 | -24 | -52 | -67 | 18 | 259 | 1773 | 5512 | 9367 | 16276 | 21123 | 36166 |
| 20 | 21 | 0 | -18 | -15 | -37 | 37 | 369 | 1224 | 5054 | 11366 | 15721 | 20338 | 23107 | 35974 |
| 20 | 22 | 0 | -9 | -34 | -70 | -95 | -76 | -55 | 101 | -64 | -92 | -119 | -125 | 91 |
| 20 | 23 | 0 | -27 | -39 | -91 | -131 | -103 | -70 | 67 | -79 | -107 | -171 | -143 | 43 |
| 20 | 24 | 0 | -65 | -55 | -98 | -116 | -110 | -86 | 64 | -80 | -116 | -184 | -171 | 51 |
| 20 | 25 | 0 | -15 | -27 | -40 | 40 | 379 | 1737 | 6736 | 14189 | 18334 | 22759 | 24865 | 37488 |
| 20 | 26 | 0 | -28 | -49 | -89 | -107 | -89 | -89 | 79 | -70 | -101 | -153 | -156 | 58 |
| 20 | 27 | 0 | -39 | -64 | -100 | -125 | -113 | -79 | 101 | -85 | -131 | -152 | -125 | 74 |
| 20 | 28 | 0 | -40 | -30 | -67 | -67 | 67 | 455 | 2204 | 5872 | 9263 | 15843 | 20696 | 35767 |
| 20 | 29 | 0 | -40 | -40 | -30 | 86 | 403 | 1697 | 5878 | 12419 | 16557 | 20909 | 23430 | 35925 |
| 20 | 30 | 0 | -31 | -25 | -58 | -25 | 152 | 824 | 3516 | 9571 | 14042 | 19291 | 22426 | 35464 |
| 20 | 31 | 0 | -21 | -12 | -21 | 226 | 537 | 1779 | 4755 | 10307 | 14253 | 19258 | 22151 | 35339 |
| 20 | 32 | 0 | -34 | -37 | -58 | 54 | 274 | 1596 | 5460 | 12867 | 17164 | 21290 | 24043 | 36529 |
| 10 | 1 | 0 | -36 | -39 | -106 | -122 | -88 | -85 | 67 | -67 | -122 | -168 | -143 | 40 |
| 10 | 2 | 0 | -40 | -43 | -88 | -110 | -92 | -92 | 61 | -85 | -116 | -174 | -146 | 73 |
| 10 | 3 | 0 | -40 | -40 | -64 | -107 | -70 | 3 | 299 | 418 | 586 | 1306 | 2646 | 29629 |
| 10 | 4 | 0 | -48 | -48 | -94 | -116 | -94 | -79 | 77 | -73 | -109 | -161 | -119 | 89 |
| 10 | 5 | 0 | -31 | -15 | -58 | 6 | 119 | 647 | 2329 | 6440 | 9977 | 16292 | 20085 | 35568 |
| 10 | 6 | 0 | -16 | -37 | -80 | -98 | -98 | -71 | 70 | -43 | -89 | -159 | -119 | 85 |
| 10 | 7 | 0 | -27 | -46 | -70 | -104 | -110 | -61 | 70 | -76 | -100 | -158 | -149 | 77 |
| 10 | 8 | 0 | -40 | -65 | -107 | -138 | -116 | -95 | 82 | -89 | -113 | -184 | -156 | 61 |
| 10 | 9 | 0 | -33 | -33 | -70 | -103 | -94 | -67 | 80 | -61 | -94 | -165 | -146 | 55 |
| 10 | 10 | 0 | -37 | -31 | -79 | -67 | 64 | 342 | 1401 | 3488 | 5274 | 10194 | 15287 | 34793 |
| 10 | 11 | 0 | -43 | -43 | -79 | -110 | -98 | -73 | 79 | -67 | -107 | -159 | -107 | 92 |
| 10 | 12 | 0 | -12 | 9 | 30 | 216 | 638 | 2197 | 6504 | 12766 | 16832 | 21150 | 24541 | 35891 |
| 10 | 13 | 0 | -37 | -19 | -40 | -25 | 195 | 906 | 3449 | 8408 | 11820 | 17363 | 20857 | 35589 |
| 10 | 14 | 0 | -12 | -3 | -25 | 24 | 271 | 1483 | 5554 | 12928 | 17231 | 21443 | 23760 | 35900 |
| 10 | 15 | 0 | -21 | -40 | -79 | -98 | -82 | 9 | 452 | 1758 | 3486 | 9693 | 15532 | 34866 |
| 10 | 16 | 0 | -39 | -45 | -100 | -88 | -76 | 6 | 412 | 1673 | 3464 | 8757 | 14458 | 36160 |
| 10 | 17 | 0 | -39 | -48 | -94 | -119 | -94 | -73 | 77 | -64 | -103 | -158 | -143 | 52 |
| 10 | 18 | 0 | -15 | -25 | -76 | -95 | -55 | -61 | 107 | -49 | -89 | -128 | -101 | 101 |
| 10 | 19 | 0 | -21 | -18 | -43 | 24 | 253 | 977 | 3241 | 6473 | 9083 | 13780 | 18455 | 34695 |
| 10 | 20 | 0 | -27 | -27 | -76 | -110 | -88 | -58 | 86 | -61 | -94 | -140 | -110 | 95 |
| 10 | 21 | 0 | -77 | -101 | -113 | -89 | 27 | 598 | 2991 | 9598 | 14369 | 19581 | 22328 | 34890 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. cloacae* NCTC 11936 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -21 | -31 | -79 | -85 | -49 | 21 | 415 | 1254 | 2323 | 6092 | 11387 | 34488 |
| 10 | 23 | 0 | -30 | -67 | -85 | -94 | -104 | -64 | 67 | -70 | -104 | -174 | -143 | 70 |
| 10 | 24 | 0 | -52 | -58 | -83 | -74 | 24 | 537 | 2624 | 8603 | 13245 | 18959 | 22325 | 36190 |
| 10 | 25 | 0 | -27 | -61 | -88 | -122 | -97 | -85 | 71 | -85 | -122 | -189 | -155 | 71 |
| 10 | 26 | 0 | -42 | -55 | -88 | -125 | -110 | -91 | 64 | -91 | -116 | -177 | -143 | 40 |
| 10 | 27 | 0 | -21 | -46 | -86 | -86 | -95 | -70 | 82 | -76 | -98 | -159 | -116 | 52 |
| 10 | 28 | 0 | -36 | -61 | -76 | -116 | -110 | -70 | 64 | -79 | -116 | -183 | -155 | 37 |
| 10 | 29 | 0 | -19 | -34 | -58 | -74 | -83 | -37 | 97 | -52 | -92 | -141 | -122 | 70 |
| 10 | 30 | 0 | -37 | -40 | -88 | -110 | -107 | -85 | 76 | -94 | -125 | -186 | -146 | 52 |
| 10 | 31 | 0 | -42 | -45 | -103 | -103 | -122 | -91 | 40 | -79 | -122 | -192 | -170 | 46 |
| 10 | 32 | 0 | -52 | -73 | -125 | -98 | -67 | 113 | 574 | 1181 | 1868 | 4032 | 6797 | 31466 |
| 5 | 1 | 0 | -43 | -71 | -86 | -89 | -22 | 204 | 827 | 1648 | 2200 | 3424 | 4852 | 33794 |
| 5 | 2 | 0 | -52 | -68 | -104 | -129 | -104 | -104 | 54 | -92 | -126 | -181 | -150 | 15 |
| 5 | 3 | 0 | -58 | -55 | -91 | -107 | -104 | -73 | 67 | -85 | -131 | -183 | -128 | 37 |
| 5 | 4 | 0 | -30 | -52 | -97 | -116 | -79 | -85 | 52 | -85 | -134 | -180 | -164 | 40 |
| 5 | 5 | 0 | -28 | -43 | -82 | -116 | -95 | -73 | 73 | -73 | -98 | -174 | -147 | 55 |
| 5 | 6 | 0 | -36 | -55 | -91 | -39 | 144 | 614 | 1877 | 3211 | 4322 | 6367 | 8607 | 32870 |
| 5 | 7 | 0 | -49 | -52 | -91 | -116 | -97 | -73 | 64 | -52 | -103 | -177 | -161 | 49 |
| 5 | 8 | 0 | -49 | -52 | -98 | -104 | -95 | 15 | 473 | 1926 | 3836 | 8896 | 14848 | 35385 |
| 5 | 9 | 0 | -15 | -48 | -85 | -116 | -97 | -76 | 83 | -79 | -122 | -180 | -137 | 86 |
| 5 | 10 | 0 | -43 | -73 | -98 | -43 | 70 | 345 | 1251 | 1895 | 2567 | 4080 | 5787 | 32876 |
| 5 | 11 | 0 | -30 | -76 | -88 | -131 | -109 | -103 | 58 | -88 | -119 | -195 | -152 | 31 |
| 5 | 12 | 0 | -33 | -36 | -91 | -125 | -125 | -88 | 58 | -85 | -119 | -180 | -167 | 34 |
| 5 | 13 | 0 | -39 | -42 | -94 | -119 | -103 | -70 | 61 | -73 | -116 | -183 | -165 | 37 |
| 5 | 14 | 0 | -46 | -49 | -94 | -119 | -91 | -70 | 73 | -73 | -110 | -174 | -162 | 49 |
| 5 | 15 | 0 | -58 | -70 | -115 | -115 | -115 | -94 | 55 | -91 | -109 | -189 | -207 | 52 |
| 5 | 16 | 0 | -67 | -83 | -134 | -147 | -122 | -119 | 30 | -107 | -156 | -229 | -168 | 15 |
| 5 | 17 | 0 | -55 | -58 | -92 | -119 | -116 | -92 | 42 | -92 | -135 | -180 | -168 | 21 |
| 5 | 18 | 0 | -70 | -73 | -107 | -143 | -119 | -119 | 46 | -119 | -128 | -207 | -171 | 28 |
| 5 | 19 | 0 | -39 | -61 | -97 | -103 | -94 | -73 | 71 | -94 | -119 | -186 | -152 | 43 |
| 5 | 20 | 0 | -18 | -33 | -85 | -109 | -100 | -64 | 86 | -82 | -113 | -177 | -137 | 46 |
| 5 | 21 | 0 | -64 | -67 | -101 | -135 | -119 | -101 | 46 | -110 | -131 | -199 | -186 | -6 |
| 5 | 22 | 0 | -24 | -39 | -91 | -107 | -97 | -70 | 92 | -61 | -100 | -174 | -143 | 43 |
| 5 | 23 | 0 | -64 | -67 | -98 | -71 | -9 | 311 | 1620 | 4645 | 7566 | 14011 | 18379 | 34762 |
| 5 | 24 | 0 | -58 | -55 | -104 | -125 | -110 | -95 | 79 | -89 | -125 | -192 | -171 | 27 |
| 5 | 25 | 0 | -42 | -58 | -103 | -134 | -67 | -85 | 77 | -94 | -125 | -167 | -140 | 43 |
| 5 | 26 | 0 | -42 | -67 | -85 | -113 | -94 | -79 | 80 | -76 | -116 | -171 | -128 | 46 |
| 5 | 27 | 0 | -82 | -125 | -146 | -159 | -122 | -113 | 31 | -116 | -165 | -217 | -177 | 12 |
| 5 | 28 | 0 | -52 | -37 | -89 | -126 | -95 | -92 | 76 | -86 | -126 | -184 | -135 | 45 |
| 5 | 29 | 0 | -46 | -68 | -101 | -147 | -113 | -107 | 61 | -101 | -119 | -187 | -171 | 39 |
| 5 | 30 | 0 | -36 | -36 | -97 | -113 | -103 | -79 | 80 | -79 | -97 | -192 | -155 | 52 |
| 5 | 31 | 0 | -58 | -67 | -113 | -150 | -125 | -98 | 64 | -104 | -132 | -180 | -165 | 33 |
| 5 | 32 | 0 | -76 | -88 | -137 | -137 | -134 | -116 | 34 | -128 | -149 | -210 | -207 | 18 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. coli* NCIMB 10213 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -250 | -339 | -360 | -305 | -314 | -320 | -375 | -275 | -317 | -305 | -305 | -180 |
| 20 | 2 | 0 | -235 | -275 | -339 | -308 | -305 | -287 | -363 | -254 | -284 | -284 | -293 | -180 |
| 20 | 3 | 0 | -244 | -309 | -333 | -287 | -293 | -278 | -379 | -293 | -296 | -305 | -290 | -202 |
| 20 | 4 | 0 | -232 | -321 | -348 | -293 | -305 | -318 | -376 | -281 | -299 | -305 | -302 | -189 |
| 20 | 5 | 0 | -229 | -324 | -366 | -305 | -278 | -293 | -385 | -269 | -241 | -241 | -287 | -177 |
| 20 | 6 | 0 | -226 | -302 | -312 | -309 | -306 | -315 | -379 | -284 | -321 | -324 | -315 | -162 |
| 20 | 7 | 0 | -244 | -339 | -360 | -324 | -324 | -324 | -388 | -275 | -330 | -324 | -330 | -235 |
| 20 | 8 | 0 | -241 | -308 | -357 | -324 | -330 | -317 | -403 | -305 | -327 | -342 | -336 | -253 |
| 20 | 9 | 0 | -235 | -308 | -351 | -290 | -317 | -302 | -381 | -275 | -299 | -296 | -272 | -156 |
| 20 | 10 | 0 | -238 | -296 | -342 | -275 | -296 | -284 | -309 | -269 | -278 | -260 | -281 | -171 |
| 20 | 11 | 0 | -232 | -305 | -345 | -305 | -302 | -293 | -363 | -275 | -281 | -287 | -275 | -205 |
| 20 | 12 | 0 | -250 | -320 | -357 | -314 | -314 | -314 | -394 | -275 | -305 | -323 | -317 | -229 |
| 20 | 13 | 0 | -232 | -287 | -330 | -281 | -290 | -275 | -357 | -260 | -272 | -269 | -278 | -211 |
| 20 | 14 | 0 | -225 | -296 | -326 | -280 | -293 | -296 | -357 | -256 | -280 | -283 | -283 | -189 |
| 20 | 15 | 0 | -210 | -299 | -323 | -292 | -286 | -286 | -341 | -244 | -283 | -277 | -289 | -213 |
| 20 | 16 | 0 | -235 | -320 | -333 | -308 | -320 | -317 | -378 | -278 | -317 | -320 | -314 | -217 |
| 20 | 17 | 0 | -235 | -311 | -329 | -287 | -296 | -287 | -354 | -275 | -287 | -275 | -284 | -168 |
| 20 | 18 | 0 | -265 | -348 | -375 | -308 | -332 | -311 | -393 | -302 | -314 | -305 | -329 | -201 |
| 20 | 19 | 0 | -235 | -296 | -336 | -296 | -293 | -275 | -348 | -269 | -278 | -281 | -299 | -217 |
| 20 | 20 | 0 | -247 | -317 | -360 | -302 | -308 | -299 | -375 | -278 | -305 | -308 | -299 | -217 |
| 20 | 21 | 0 | -214 | -299 | -339 | -290 | -290 | -296 | -348 | -257 | -278 | -269 | -293 | -199 |
| 20 | 22 | 0 | -229 | -315 | -354 | -293 | -293 | -299 | -366 | -272 | -290 | -302 | -296 | -202 |
| 20 | 23 | 0 | -204 | -272 | -293 | -250 | -272 | -259 | -330 | -223 | -253 | -241 | -250 | -180 |
| 20 | 24 | 0 | -241 | -339 | -345 | -305 | -308 | -302 | -372 | -284 | -308 | -290 | -311 | -223 |
| 20 | 25 | 0 | -253 | -339 | -354 | -314 | -326 | -305 | -400 | -308 | -305 | -296 | -299 | -201 |
| 20 | 26 | 0 | -235 | -314 | -345 | -293 | -320 | -308 | -369 | -281 | -287 | -278 | -302 | -189 |
| 20 | 27 | 0 | -232 | -302 | -348 | -281 | -293 | -284 | -370 | -257 | -278 | -263 | -284 | -193 |
| 20 | 28 | 0 | -250 | -339 | -372 | -323 | -335 | -329 | -397 | -299 | -311 | -305 | -293 | -235 |
| 20 | 29 | 0 | -210 | -286 | -329 | -268 | -299 | -286 | -351 | -235 | -253 | -250 | -265 | -183 |
| 20 | 30 | 0 | -204 | -284 | -320 | -274 | -271 | -287 | -351 | -250 | -271 | -284 | -271 | -180 |
| 20 | 31 | 0 | -217 | -300 | -327 | -290 | -300 | -278 | -361 | -263 | -281 | -275 | -287 | -187 |
| 20 | 32 | 0 | -235 | -324 | -345 | -302 | -302 | -290 | -348 | -275 | -299 | -308 | -299 | -220 |
| 10 | 1 | 0 | -229 | -317 | -351 | -293 | -317 | -302 | -379 | -284 | -284 | -293 | -302 | -192 |
| 10 | 2 | 0 | -223 | -312 | -342 | -275 | -308 | -284 | -373 | -272 | -275 | -275 | -287 | -199 |
| 10 | 3 | 0 | -251 | -309 | -339 | -278 | -309 | -300 | -351 | -269 | -293 | -290 | -281 | -217 |
| 10 | 4 | 0 | -247 | -305 | -333 | -284 | -296 | -290 | -373 | -257 | -302 | -287 | -293 | -217 |
| 10 | 5 | 0 | -248 | -309 | -342 | -302 | -293 | -299 | -348 | -293 | -299 | -281 | -296 | -205 |
| 10 | 6 | 0 | -220 | -308 | -323 | -293 | -299 | -287 | -339 | -256 | -271 | -284 | -274 | -189 |
| 10 | 7 | 0 | -201 | -296 | -323 | -268 | -290 | -277 | -348 | -219 | -259 | -271 | -271 | -192 |
| 10 | 8 | 0 | -244 | -330 | -351 | -302 | -305 | -302 | -354 | -272 | -308 | -296 | -302 | -214 |
| 10 | 9 | 0 | -238 | -314 | -351 | -302 | -305 | -299 | -385 | -266 | -293 | -299 | -290 | -186 |
| 10 | 10 | 0 | -248 | -312 | -345 | -302 | -305 | -275 | -348 | -248 | -266 | -272 | -272 | -190 |
| 10 | 11 | 0 | -238 | -318 | -339 | -275 | -312 | -296 | -373 | -275 | -318 | -287 | -281 | -214 |
| 10 | 12 | 0 | -201 | -287 | -299 | -272 | -275 | -262 | -336 | -238 | -241 | -253 | -259 | -180 |
| 10 | 13 | 0 | -226 | -305 | -326 | -274 | -290 | -274 | -335 | -250 | -265 | -262 | -268 | -186 |
| 10 | 14 | 0 | -210 | -308 | -320 | -268 | -296 | -271 | -348 | -250 | -274 | -281 | -265 | -192 |
| 10 | 15 | 0 | -210 | -287 | -326 | -274 | -274 | -271 | -335 | -250 | -268 | -253 | -268 | -207 |
| 10 | 16 | 0 | -226 | -312 | -345 | -293 | -302 | -290 | -360 | -275 | -296 | -287 | -296 | -190 |
| 10 | 17 | 0 | -253 | -342 | -366 | -293 | -342 | -311 | -406 | -278 | -305 | -323 | -287 | -192 |
| 10 | 18 | 0 | -229 | -351 | -345 | -299 | -290 | -293 | -367 | -275 | -284 | -275 | -272 | -171 |
| 10 | 19 | 0 | -239 | -315 | -324 | -290 | -303 | -293 | -361 | -269 | -275 | -287 | -278 | -205 |
| 10 | 20 | 0 | -231 | -299 | -323 | -277 | -289 | -292 | -363 | -256 | -277 | -289 | -277 | -213 |
| 10 | 21 | 0 | -222 | -305 | -344 | -292 | -292 | -274 | -354 | -256 | -283 | -283 | -280 | -204 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. coli* NCIMB 10213 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -216 | -296 | -314 | -290 | -290 | -268 | -339 | -256 | -271 | -274 | -265 | -192 |
| 10 | 23 | 0 | -216 | -311 | -320 | -277 | -277 | -262 | -345 | -241 | -265 | -274 | -274 | -155 |
| 10 | 24 | 0 | -232 | -296 | -342 | -284 | -296 | -281 | -363 | -260 | -278 | -272 | -281 | -180 |
| 10 | 25 | 0 | -238 | -339 | -348 | -305 | -275 | -302 | -388 | -305 | -290 | -305 | -272 | -201 |
| 10 | 26 | 0 | -211 | -306 | -315 | -284 | -287 | -281 | -361 | -269 | -266 | -287 | -272 | -193 |
| 10 | 27 | 0 | -226 | -305 | -345 | -283 | -290 | -287 | -357 | -256 | -283 | -271 | -277 | -192 |
| 10 | 28 | 0 | -242 | -303 | -336 | -287 | -281 | -281 | -324 | -229 | -272 | -251 | -284 | -202 |
| 10 | 29 | 0 | -219 | -302 | -314 | -284 | -290 | -277 | -354 | -250 | -268 | -271 | -265 | -195 |
| 10 | 30 | 0 | -235 | -326 | -320 | -290 | -311 | -296 | -348 | -265 | -278 | -299 | -281 | -201 |
| 10 | 31 | 0 | -241 | -330 | -354 | -299 | -305 | -290 | -370 | -284 | -299 | -296 | -293 | -189 |
| 10 | 32 | 0 | -220 | -299 | -339 | -278 | -312 | -296 | -370 | -263 | -287 | -275 | -272 | -162 |
| 5 | 1 | 0 | -228 | -293 | -332 | -271 | -286 | -271 | -335 | -253 | -259 | -280 | -268 | -164 |
| 5 | 2 | 0 | -213 | -284 | -305 | -271 | -284 | -274 | -332 | -244 | -238 | -262 | -253 | -140 |
| 5 | 3 | 0 | -238 | -305 | -339 | -302 | -293 | -299 | -348 | -260 | -278 | -281 | -278 | -190 |
| 5 | 4 | 0 | -222 | -302 | -320 | -256 | -277 | -280 | -348 | -244 | -271 | -274 | -265 | -195 |
| 5 | 5 | 0 | -232 | -311 | -335 | -299 | -299 | -289 | -354 | -274 | -280 | -293 | -280 | -222 |
| 5 | 6 | 0 | -251 | -333 | -360 | -302 | -321 | -296 | -382 | -284 | -308 | -308 | -287 | -202 |
| 5 | 7 | 0 | -239 | -309 | -321 | -296 | -284 | -284 | -351 | -272 | -260 | -287 | -303 | -193 |
| 5 | 8 | 0 | -251 | -321 | -357 | -299 | -312 | -315 | -379 | -266 | -287 | -284 | -260 | -165 |
| 5 | 9 | 0 | -250 | -333 | -354 | -308 | -345 | -317 | -372 | -281 | -305 | -314 | -293 | -198 |
| 5 | 10 | 0 | -238 | -324 | -339 | -308 | -299 | -302 | -348 | -272 | -296 | -296 | -293 | -195 |
| 5 | 11 | 0 | -241 | -289 | -323 | -289 | -296 | -268 | -357 | -250 | -271 | -271 | -268 | -192 |
| 5 | 12 | 0 | -245 | -306 | -342 | -284 | -299 | -290 | -348 | -251 | -281 | -278 | -287 | -196 |
| 5 | 13 | 0 | -226 | -283 | -335 | -283 | -293 | -277 | -366 | -247 | -265 | -268 | -268 | -207 |
| 5 | 14 | 0 | -251 | -312 | -364 | -293 | -290 | -287 | -364 | -251 | -287 | -303 | -278 | -199 |
| 5 | 15 | 0 | -229 | -315 | -351 | -287 | -308 | -305 | -379 | -275 | -284 | -281 | -287 | -223 |
| 5 | 16 | 0 | -244 | -326 | -375 | -302 | -320 | -305 | -369 | -287 | -296 | -296 | -299 | -180 |
| 5 | 17 | 0 | -238 | -311 | -342 | -269 | -299 | -287 | -360 | -266 | -287 | -296 | -293 | -171 |
| 5 | 18 | 0 | -251 | -305 | -330 | -293 | -312 | -299 | -382 | -260 | -290 | -287 | -281 | -177 |
| 5 | 19 | 0 | -210 | -280 | -320 | -305 | -308 | -271 | -341 | -247 | -277 | -274 | -277 | -171 |
| 5 | 20 | 0 | -235 | -306 | -336 | -278 | -300 | -281 | -358 | -257 | -275 | -284 | -281 | -196 |
| 5 | 21 | 0 | -226 | -296 | -323 | -277 | -296 | -277 | -345 | -265 | -259 | -280 | -265 | -180 |
| 5 | 22 | 0 | -232 | -305 | -336 | -281 | -290 | -268 | -345 | -244 | -259 | -281 | -268 | -177 |
| 5 | 23 | 0 | -238 | -314 | -351 | -293 | -305 | -308 | -372 | -275 | -290 | -302 | -293 | -199 |
| 5 | 24 | 0 | -223 | -314 | -360 | -327 | -299 | -290 | -376 | -281 | -287 | -296 | -269 | -183 |
| 5 | 25 | 0 | -247 | -345 | -351 | -327 | -327 | -311 | -382 | -293 | -302 | -311 | -305 | -177 |
| 5 | 26 | 0 | -229 | -312 | -333 | -284 | -287 | -324 | -373 | -247 | -284 | -290 | -287 | -156 |
| 5 | 27 | 0 | -259 | -339 | -354 | -308 | -324 | -308 | -385 | -287 | -296 | -317 | -302 | -189 |
| 5 | 28 | 0 | -229 | -336 | -351 | -305 | -314 | -311 | -366 | -259 | -293 | -311 | -287 | -208 |
| 5 | 29 | 0 | -238 | -312 | -348 | -296 | -302 | -312 | -364 | -278 | -284 | -266 | -278 | -180 |
| 5 | 30 | 0 | -229 | -308 | -345 | -287 | -305 | -284 | -373 | -266 | -287 | -296 | -266 | -174 |
| 5 | 31 | 0 | -211 | -306 | -339 | -287 | -293 | -284 | -364 | -257 | -269 | -287 | -275 | -162 |
| 5 | 32 | 0 | -223 | -293 | -345 | -269 | -312 | -287 | -357 | -266 | -287 | -293 | -266 | -171 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. aerogenes* NCIMB 10102 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -168 | -223 | -293 | -116 | 244 | 1733 | 4202 | 8240 | 13349 | 16532 | 24675 | 37030 |
| 20 | 2 | 0 | -183 | -229 | -302 | -226 | -192 | 79 | 452 | 1514 | 4169 | 7996 | 15751 | 35898 |
| 20 | 3 | 0 | -196 | -257 | -336 | -248 | -260 | -113 | 616 | 2615 | 6687 | 10816 | 20082 | 35128 |
| 20 | 4 | 0 | -223 | -269 | -342 | -269 | -275 | -208 | 104 | 1309 | 4148 | 7432 | 15391 | 33895 |
| 20 | 5 | 0 | -253 | -281 | -345 | -250 | -308 | -220 | -40 | 821 | 2845 | 5539 | 12397 | 34323 |
| 20 | 6 | 0 | -226 | -278 | -339 | -254 | -299 | -205 | 76 | 1129 | 3412 | 6223 | 13093 | 34896 |
| 20 | 7 | 0 | -184 | -263 | -339 | -257 | -275 | -242 | -263 | -141 | 274 | 1291 | 4053 | 31350 |
| 20 | 8 | 0 | -214 | -287 | -336 | -253 | -302 | -250 | -235 | -34 | 583 | 1737 | 4297 | 31524 |
| 20 | 9 | 0 | -135 | -217 | -251 | -92 | 235 | 1697 | 4395 | 7816 | 11979 | 15363 | 23039 | 35922 |
| 20 | 10 | 0 | -126 | -223 | -278 | -174 | -71 | 631 | 2133 | 4816 | 8472 | 12305 | 20112 | 34615 |
| 20 | 11 | 0 | -146 | -195 | -274 | -171 | -119 | 348 | 1322 | 3327 | 6547 | 9901 | 18202 | 35117 |
| 20 | 12 | 0 | -131 | -192 | -259 | -171 | -198 | -46 | 226 | 1017 | 2961 | 5396 | 12172 | 33731 |
| 20 | 13 | 0 | -162 | -205 | -272 | -196 | -220 | -71 | 170 | 1043 | 2969 | 5566 | 12430 | 33721 |
| 20 | 14 | 0 | -125 | -186 | -256 | -174 | -180 | -15 | 263 | 1145 | 3250 | 5921 | 12828 | 33548 |
| 20 | 15 | 0 | -116 | -195 | -262 | -186 | -195 | -128 | 52 | 797 | 2601 | 5146 | 10817 | 32556 |
| 20 | 16 | 0 | -140 | -204 | -253 | -186 | -231 | -164 | -158 | 150 | 1108 | 2695 | 6632 | 31125 |
| 20 | 17 | 0 | -153 | -205 | -257 | -186 | -74 | 845 | 2695 | 5832 | 9647 | 13319 | 20671 | 35839 |
| 20 | 18 | 0 | -155 | -198 | -253 | -158 | -64 | 467 | 1490 | 3294 | 6321 | 9245 | 16442 | 34189 |
| 20 | 19 | 0 | -140 | -171 | -268 | -155 | -67 | 571 | 1847 | 4185 | 7505 | 10725 | 18694 | 33740 |
| 20 | 20 | 0 | -125 | -183 | -241 | -180 | -52 | 644 | 2250 | 5128 | 9309 | 12730 | 21383 | 34094 |
| 20 | 21 | 0 | -173 | -207 | -262 | -192 | -122 | 351 | 1203 | 3202 | 5897 | 9166 | 17604 | 33719 |
| 20 | 22 | 0 | -140 | -186 | -278 | -174 | -122 | 186 | 733 | 2069 | 4688 | 7694 | 15437 | 33496 |
| 20 | 23 | 0 | -119 | -174 | -229 | -174 | -189 | -107 | 3 | 424 | 1587 | 3620 | 8607 | 32437 |
| 20 | 24 | 0 | -119 | -195 | -238 | -162 | -183 | -36 | 220 | 1068 | 3089 | 5945 | 12602 | 33053 |
| 20 | 25 | 0 | -134 | -201 | -250 | -128 | 92 | 1084 | 2988 | 5839 | 9559 | 13051 | 20571 | 34573 |
| 20 | 26 | 0 | -122 | -201 | -247 | -110 | 49 | 766 | 2076 | 4294 | 7624 | 11061 | 17726 | 33642 |
| 20 | 27 | 0 | -140 | -177 | -241 | -146 | -36 | 788 | 2311 | 4819 | 8277 | 10990 | 18446 | 33813 |
| 20 | 28 | 0 | -134 | -204 | -271 | -174 | -158 | 296 | 1313 | 3419 | 6556 | 9916 | 17907 | 33453 |
| 20 | 29 | 0 | -143 | -189 | -247 | -192 | -186 | 76 | 580 | 1755 | 3800 | 6611 | 13841 | 33111 |
| 20 | 30 | 0 | -116 | -195 | -259 | -183 | -189 | -61 | 192 | 885 | 2451 | 4685 | 10850 | 32583 |
| 20 | 31 | 0 | -110 | -192 | -253 | -159 | -171 | -70 | 253 | 995 | 2863 | 5542 | 11652 | 32476 |
| 20 | 32 | 0 | -137 | -222 | -271 | -180 | -143 | 147 | 574 | 1652 | 3822 | 6660 | 13396 | 32837 |
| 10 | 1 | 0 | -104 | -168 | -238 | -122 | -40 | 531 | 1666 | 3794 | 6623 | 9928 | 16841 | 34012 |
| 10 | 2 | 0 | -128 | -192 | -250 | -161 | -73 | 715 | 2411 | 5079 | 8607 | 12416 | 19124 | 33728 |
| 10 | 3 | 0 | -131 | -180 | -254 | -168 | -177 | 94 | 818 | 2255 | 4981 | 7364 | 14158 | 32830 |
| 10 | 4 | 0 | -128 | -210 | -259 | -168 | -110 | 336 | 1288 | 3122 | 6165 | 9040 | 16457 | 33520 |
| 10 | 5 | 0 | -149 | -192 | -250 | -192 | -168 | 58 | 602 | 1749 | 3641 | 6214 | 13243 | 32907 |
| 10 | 6 | 0 | -113 | -165 | -229 | -168 | -159 | 88 | 622 | 1810 | 4193 | 7062 | 14195 | 32491 |
| 10 | 7 | 0 | -125 | -189 | -259 | -165 | -122 | 137 | 702 | 1883 | 4096 | 7398 | 14451 | 32534 |
| 10 | 8 | 0 | -125 | -195 | -238 | -180 | -155 | 86 | 418 | 1154 | 2695 | 5128 | 11045 | 32879 |
| 10 | 9 | 0 | -146 | -192 | -241 | -158 | -73 | 583 | 1865 | 4093 | 7237 | 10927 | 18370 | 33389 |
| 10 | 10 | 0 | -140 | -186 | -238 | -168 | -162 | 83 | 513 | 1325 | 3073 | 5884 | 10758 | 33200 |
| 10 | 11 | 0 | -119 | -171 | -247 | -156 | -107 | 400 | 1715 | 4218 | 7843 | 10786 | 18175 | 33413 |
| 10 | 12 | 0 | -155 | -204 | -262 | -155 | -122 | 290 | 1078 | 2652 | 5671 | 8213 | 15660 | 32931 |
| 10 | 13 | 0 | -134 | -192 | -269 | -174 | -183 | -156 | -202 | -137 | -214 | -253 | -92 | -9 |
| 10 | 14 | 0 | -137 | -189 | -229 | -168 | -125 | 330 | 1206 | 3104 | 6019 | 9046 | 16621 | 32867 |
| 10 | 15 | 0 | -104 | -180 | -247 | -174 | -165 | 40 | 510 | 1581 | 3580 | 6421 | 12946 | 32177 |
| 10 | 16 | 0 | -125 | -174 | -247 | -165 | -198 | -49 | 140 | 775 | 2103 | 4230 | 9629 | 32659 |
| 10 | 17 | 0 | -128 | -201 | -265 | -171 | -131 | 196 | 623 | 1273 | 2100 | 3058 | 6159 | 31964 |
| 10 | 18 | 0 | -128 | -183 | -262 | -183 | -201 | -46 | 138 | 592 | 1575 | 3589 | 7691 | 32473 |
| 10 | 19 | 0 | -137 | -189 | -253 | -159 | -116 | 134 | 418 | 928 | 1578 | 2243 | 4294 | 31494 |
| 10 | 20 | 0 | -134 | -220 | -265 | -177 | -143 | 379 | 1676 | 3800 | 7520 | 10679 | 17693 | 33084 |
| 10 | 21 | 0 | -146 | -201 | -275 | -198 | -204 | -171 | -183 | -149 | -226 | -275 | -101 | -34 |

**Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.**

*E. aerogenes* NCIMB 10102 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -125 | -217 | -268 | -171 | -143 | 369 | 1416 | 3467 | 6623 | 9611 | 16963 | 32867 |
| 10 | 23 | 0 | -101 | -177 | -244 | -153 | -134 | 49 | 461 | 1453 | 3262 | 5689 | 11326 | 31976 |
| 10 | 24 | 0 | -122 | -180 | -256 | -177 | -201 | -156 | -183 | -149 | -217 | -232 | -107 | -30 |
| 10 | 25 | 0 | -128 | -174 | -241 | -158 | -174 | -33 | 141 | 543 | 1474 | 3077 | 7447 | 33203 |
| 10 | 26 | 0 | -125 | -171 | -238 | -171 | -195 | -67 | 266 | 1196 | 3370 | 6480 | 12602 | 32980 |
| 10 | 27 | 0 | -134 | -165 | -247 | -128 | -58 | 497 | 1682 | 3894 | 7105 | 10709 | 17106 | 32794 |
| 10 | 28 | 0 | -161 | -189 | -253 | -183 | -131 | 254 | 977 | 2424 | 5207 | 8134 | 14580 | 33115 |
| 10 | 29 | 0 | -135 | -177 | -223 | -86 | 134 | 1352 | 3412 | 6037 | 9757 | 12501 | 19777 | 32760 |
| 10 | 30 | 0 | -116 | -177 | -241 | -159 | -141 | 30 | 223 | 622 | 1117 | 1562 | 3238 | 30620 |
| 10 | 31 | 0 | -113 | -177 | -241 | -147 | -144 | 207 | 1007 | 2701 | 5335 | 8524 | 15181 | 32635 |
| 10 | 32 | 0 | -98 | -159 | -201 | -67 | 76 | 848 | 1953 | 4065 | 7123 | 10542 | 17860 | 33514 |
| 5 | 1 | 0 | -144 | -199 | -266 | -193 | -211 | -64 | 167 | 625 | 1913 | 3952 | 9030 | 32571 |
| 5 | 2 | 0 | -131 | -189 | -238 | -174 | -192 | -79 | 211 | 974 | 3013 | 5775 | 11509 | 32742 |
| 5 | 3 | 0 | -140 | -180 | -247 | -162 | -207 | -156 | -189 | -149 | -204 | -262 | -104 | -18 |
| 5 | 4 | 0 | -128 | -177 | -238 | -171 | -180 | 21 | 507 | 1770 | 4273 | 7633 | 13615 | 32852 |
| 5 | 5 | 0 | -132 | -174 | -238 | -159 | -193 | -135 | -171 | -132 | -193 | -232 | -67 | -3 |
| 5 | 6 | 0 | -122 | -180 | -241 | -165 | -214 | -104 | 235 | 1077 | 3055 | 5399 | 11518 | 32223 |
| 5 | 7 | 0 | -119 | -186 | -251 | -171 | -177 | -98 | 73 | 711 | 2164 | 4242 | 9147 | 31930 |
| 5 | 8 | 0 | -125 | -180 | -235 | -177 | -183 | 275 | 1309 | 3406 | 6617 | 10087 | 17302 | 33719 |
| 5 | 9 | 0 | -153 | -205 | -275 | -205 | -244 | -171 | -223 | -193 | -235 | -263 | -141 | -52 |
| 5 | 10 | 0 | -125 | -189 | -235 | -174 | -168 | 46 | 370 | 1078 | 2985 | 5497 | 11119 | 32641 |
| 5 | 11 | 0 | -150 | -180 | -259 | -177 | -208 | -150 | -180 | -146 | -192 | -259 | -82 | -18 |
| 5 | 12 | 0 | -140 | -174 | -238 | -146 | -94 | 217 | 806 | 1978 | 4334 | 7346 | 13115 | 33026 |
| 5 | 13 | 0 | -140 | -186 | -259 | -177 | -195 | 116 | 906 | 2521 | 5478 | 8524 | 15095 | 32888 |
| 5 | 14 | 0 | -113 | -192 | -241 | -137 | -67 | 253 | 543 | 1086 | 1678 | 2365 | 4447 | 31515 |
| 5 | 15 | 0 | -113 | -195 | -244 | -168 | -150 | 324 | 1343 | 3308 | 6275 | 9715 | 16630 | 32656 |
| 5 | 16 | 0 | -128 | -183 | -232 | -110 | -58 | 326 | 882 | 2100 | 4093 | 6553 | 12223 | 33392 |
| 5 | 17 | 0 | -153 | -202 | -269 | -190 | -162 | 15 | 213 | 799 | 2414 | 4794 | 10206 | 31350 |
| 5 | 18 | 0 | -129 | -177 | -257 | -187 | -214 | -156 | -181 | -165 | -211 | -266 | -110 | -25 |
| 5 | 19 | 0 | -128 | -180 | -262 | -192 | -186 | -24 | 311 | 1102 | 2991 | 5814 | 11396 | 32696 |
| 5 | 20 | 0 | -167 | -198 | -268 | -149 | -116 | 266 | 767 | 1627 | 3144 | 5265 | 10353 | 33215 |
| 5 | 21 | 0 | -143 | -192 | -253 | -155 | -149 | 80 | 299 | 711 | 1145 | 1660 | 3247 | 31402 |
| 5 | 22 | 0 | -119 | -183 | -241 | -189 | -165 | 232 | 1233 | 3009 | 6043 | 9223 | 15916 | 33127 |
| 5 | 23 | 0 | -113 | -168 | -232 | -104 | 12 | 373 | 775 | 1416 | 2021 | 2927 | 5228 | 32086 |
| 5 | 24 | 0 | -149 | -198 | -232 | -94 | 25 | 528 | 1062 | 1874 | 2613 | 3376 | 5741 | 33514 |
| 5 | 25 | 0 | -167 | -213 | -287 | -229 | -232 | -158 | -219 | -201 | -244 | -299 | -167 | -91 |
| 5 | 26 | 0 | -152 | -207 | -274 | -195 | -219 | -155 | 16 | 495 | 1835 | 3873 | 8833 | 34494 |
| 5 | 27 | 0 | -131 | -177 | -238 | -180 | -202 | -153 | -198 | -140 | -205 | -256 | -122 | -31 |
| 5 | 28 | 0 | -155 | -198 | -259 | -207 | -204 | -177 | -201 | -165 | -247 | -262 | -107 | -12 |
| 5 | 29 | 0 | -180 | -222 | -283 | -219 | -195 | 242 | 1172 | 3217 | 6404 | 9434 | 16432 | 35239 |
| 5 | 30 | 0 | -153 | -217 | -269 | -168 | -131 | 143 | 403 | 839 | 1285 | 1919 | 3510 | 34848 |
| 5 | 31 | 0 | -155 | -226 | -277 | -116 | -3 | 660 | 1783 | 4227 | 7121 | 10371 | 17269 | 35339 |
| 5 | 32 | 0 | -159 | -198 | -250 | -195 | -192 | 58 | 568 | 1941 | 4593 | 7465 | 13826 | 36093 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*C. freundii* FRHCFR2 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -55 | -82 | -116 | -61 | -88 | -104 | -95 | -64 | -101 | -37 | -34 | 26620 |
| 20 | 2 | 0 | -86 | -98 | -98 | 70 | 296 | 528 | 1004 | 2319 | 3680 | 5704 | 10535 | 29387 |
| 20 | 3 | 0 | -79 | -104 | -122 | -79 | -76 | -119 | -110 | -73 | -97 | -61 | -79 | 34 |
| 20 | 4 | 0 | -79 | -79 | -128 | -82 | -76 | -82 | 135 | 1163 | 2826 | 5518 | 10588 | 29153 |
| 20 | 5 | 0 | -91 | -103 | -131 | -61 | -94 | -125 | -106 | -58 | -122 | -79 | -67 | 13 |
| 20 | 6 | 0 | -67 | -107 | -131 | -64 | -61 | 46 | 556 | 1499 | 2686 | 4404 | 8292 | 28533 |
| 20 | 7 | 0 | -58 | -101 | -110 | -43 | -34 | 95 | 604 | 1648 | 3238 | 5896 | 13276 | 27340 |
| 20 | 8 | 0 | -61 | -110 | -134 | -30 | -91 | -146 | -122 | -94 | -116 | -91 | -97 | -21 |
| 20 | 9 | 0 | -76 | -100 | -100 | -52 | -91 | -64 | 101 | 1410 | 3650 | 6571 | 12202 | 29785 |
| 20 | 10 | 0 | -88 | -116 | -134 | -70 | -67 | -94 | -21 | 492 | 2308 | 5439 | 13692 | 29086 |
| 20 | 11 | 0 | -73 | -128 | -116 | -18 | 104 | 431 | 1093 | 2253 | 4169 | 7105 | 14400 | 28686 |
| 20 | 12 | 0 | -91 | -122 | -104 | -61 | -91 | -91 | -58 | 83 | 537 | 1847 | 6376 | 29000 |
| 20 | 13 | 0 | -64 | -101 | -110 | -15 | 40 | 314 | 1764 | 6147 | 13288 | 20189 | 26351 | 28942 |
| 20 | 14 | 0 | -58 | -95 | -107 | -58 | -43 | -49 | 82 | 607 | 1856 | 4093 | 8823 | 28274 |
| 20 | 15 | 0 | -49 | -86 | -104 | -58 | -52 | -98 | -92 | -55 | -67 | -61 | -49 | 67 |
| 20 | 16 | 0 | -34 | -86 | -101 | -43 | -61 | -76 | -25 | 351 | 897 | 1776 | 3702 | 27627 |
| 20 | 17 | 0 | -82 | -107 | -101 | -46 | 64 | 427 | 1132 | 2567 | 4551 | 7523 | 11717 | 29360 |
| 20 | 18 | 0 | -67 | -92 | -113 | -61 | -70 | -110 | -95 | -70 | -79 | -43 | -49 | 46 |
| 20 | 19 | 0 | -52 | -92 | -76 | -31 | 18 | 171 | 1114 | 6476 | 13917 | 19804 | 24925 | 29088 |
| 20 | 20 | 0 | -73 | -107 | -107 | -30 | 18 | 229 | 1731 | 7331 | 15150 | 21089 | 25649 | 29434 |
| 20 | 21 | 0 | -94 | -116 | -137 | -67 | -97 | -119 | -119 | -42 | -116 | -85 | -67 | 0 |
| 20 | 22 | 0 | -49 | -98 | -116 | -70 | -64 | -110 | -98 | -46 | -76 | -49 | -58 | 34 |
| 20 | 23 | 0 | -49 | -85 | -98 | -43 | -15 | 73 | 549 | 2387 | 5942 | 11140 | 19679 | 28594 |
| 20 | 24 | 0 | -61 | -91 | -113 | -30 | 28 | 324 | 1371 | 3696 | 6431 | 10383 | 19872 | 28890 |
| 20 | 25 | 0 | -73 | -101 | -119 | -107 | -94 | -113 | -113 | -88 | -128 | -76 | -85 | -3 |
| 20 | 26 | 0 | -64 | -83 | -95 | -55 | -58 | -89 | -107 | -40 | -79 | -34 | -37 | 24 |
| 20 | 27 | 0 | -70 | -83 | -101 | -58 | -61 | -95 | -79 | -40 | -52 | -28 | -22 | 49 |
| 20 | 28 | 0 | -76 | -73 | 64 | 552 | 1291 | 2622 | 5509 | 11631 | 17766 | 22261 | 25835 | 29168 |
| 20 | 29 | 0 | -73 | -116 | -134 | -70 | -85 | -110 | -107 | -49 | -110 | -79 | -64 | -12 |
| 20 | 30 | 0 | -73 | -119 | -128 | -61 | -85 | -122 | -113 | -49 | -107 | -61 | -61 | -15 |
| 20 | 31 | 0 | -61 | -76 | -104 | -18 | 34 | 327 | 2088 | 8185 | 16423 | 22692 | 27248 | 28878 |
| 20 | 32 | 0 | -64 | -88 | -128 | -64 | -73 | -116 | -104 | -36 | -67 | -33 | -46 | 31 |
| 10 | 1 | 0 | -91 | -104 | -116 | -79 | -70 | -113 | -100 | 86 | 608 | 1545 | 2979 | 28503 |
| 10 | 2 | 0 | -92 | -113 | -113 | -79 | -82 | -92 | -85 | -70 | -92 | -31 | -55 | 12 |
| 10 | 3 | 0 | -91 | -104 | -119 | -52 | -6 | 168 | 1071 | 5546 | 12126 | 18599 | 24105 | 28545 |
| 10 | 4 | 0 | -92 | -98 | -143 | -88 | -98 | -113 | -110 | -67 | -107 | -55 | -64 | -3 |
| 10 | 5 | 0 | -79 | -113 | -134 | -70 | -70 | -110 | -107 | -64 | -104 | -64 | -49 | 6 |
| 10 | 6 | 0 | -82 | -125 | -131 | -73 | -82 | -113 | -122 | -73 | -113 | -61 | -64 | -18 |
| 10 | 7 | 0 | -100 | -131 | -137 | -88 | -128 | -122 | -110 | -64 | -85 | -73 | -73 | 6 |
| 10 | 8 | 0 | -76 | -85 | -106 | 37 | 223 | 864 | 3034 | 8711 | 16179 | 23165 | 27072 | 30005 |
| 10 | 9 | 0 | -64 | -98 | -101 | -76 | -64 | -104 | -95 | -73 | -104 | -49 | -67 | 0 |
| 10 | 10 | 0 | -61 | -92 | -116 | -70 | -64 | -95 | -98 | -55 | -70 | -55 | -46 | 12 |
| 10 | 11 | 0 | -64 | -101 | -113 | -79 | -82 | -116 | -122 | -67 | -101 | -55 | -58 | -6 |
| 10 | 12 | 0 | -89 | -110 | -116 | -67 | -70 | -101 | -98 | -61 | -95 | -49 | -43 | 0 |
| 10 | 13 | 0 | -61 | -64 | 3 | 378 | 690 | 1218 | 2649 | 8671 | 15678 | 20943 | 25179 | 28817 |
| 10 | 14 | 0 | -94 | -125 | -137 | -73 | -82 | -125 | -98 | 12 | 229 | 919 | 2057 | 26958 |
| 10 | 15 | 0 | -67 | -104 | -125 | -49 | -6 | 113 | 583 | 1935 | 4727 | 9754 | 18800 | 29470 |
| 10 | 16 | 0 | -64 | -98 | -92 | -18 | -12 | 150 | 1004 | 4621 | 11573 | 19209 | 25731 | 30258 |
| 10 | 17 | 0 | -103 | -161 | -115 | -112 | -109 | -149 | -140 | -85 | -131 | -82 | -94 | -36 |
| 10 | 18 | 0 | -70 | -113 | -107 | -61 | -30 | 116 | 797 | 2994 | 7285 | 13472 | 21114 | 29089 |
| 10 | 19 | 0 | -76 | -98 | -128 | -67 | -82 | -116 | -113 | -67 | -101 | -64 | -64 | -24 |
| 10 | 20 | 0 | -76 | -101 | -122 | -58 | -92 | -101 | -95 | -64 | -89 | -67 | -64 | -12 |
| 10 | 21 | 0 | -80 | -110 | -113 | -67 | -77 | -107 | -101 | -49 | -89 | -55 | -52 | 0 |

**Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.**

*C. freundii* FRHCFR2 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -79 | -92 | -40 | 162 | 299 | 485 | 867 | 1437 | 2042 | 3229 | 7126 | 28374 |
| 10 | 23 | 0 | -70 | -101 | -122 | -82 | -82 | -104 | -98 | -49 | -64 | -70 | -46 | 27 |
| 10 | 24 | 0 | -40 | -79 | -104 | -58 | -64 | -95 | -79 | -43 | -76 | -37 | -37 | 37 |
| 10 | 25 | 0 | -88 | -113 | -116 | -88 | -91 | -125 | -122 | -70 | -110 | -61 | -76 | -36 |
| 10 | 26 | 0 | -94 | -110 | -128 | -94 | -98 | -113 | -113 | -70 | -91 | -64 | -61 | 3 |
| 10 | 27 | 0 | -67 | -107 | -116 | -70 | -77 | -98 | -98 | -43 | -89 | -52 | -49 | -13 |
| 10 | 28 | 0 | -68 | -83 | -92 | -52 | -61 | -92 | -95 | -28 | -86 | -49 | -61 | 12 |
| 10 | 29 | 0 | -79 | -110 | -128 | -49 | -79 | -113 | -101 | -55 | -95 | -61 | -70 | -3 |
| 10 | 30 | 0 | -76 | -116 | -128 | -86 | -80 | -119 | -110 | -73 | -95 | -70 | -64 | -6 |
| 10 | 31 | 0 | -58 | -98 | -67 | 213 | 577 | 1172 | 1987 | 3113 | 4172 | 5399 | 7096 | 27025 |
| 10 | 32 | 0 | -40 | -88 | -98 | -52 | -58 | -43 | 107 | 855 | 2799 | 7349 | 15263 | 29806 |
| 5 | 1 | 0 | -103 | -116 | -116 | -94 | -97 | -131 | -122 | -70 | -103 | -67 | -76 | -21 |
| 5 | 2 | 0 | -58 | -98 | -98 | -52 | -34 | -12 | 363 | 2191 | 5658 | 10645 | 18687 | 28881 |
| 5 | 3 | 0 | -64 | -92 | -101 | -40 | -46 | -83 | -83 | -43 | -77 | -34 | -34 | -10 |
| 5 | 4 | 0 | -73 | -101 | -122 | -52 | -55 | -98 | -116 | -61 | -98 | -64 | -61 | -3 |
| 5 | 5 | 0 | -104 | -128 | -149 | -82 | -94 | -125 | -110 | -79 | -119 | -73 | -67 | -40 |
| 5 | 6 | 0 | -88 | -125 | -149 | -104 | -88 | -143 | -131 | -82 | -125 | -110 | -67 | -27 |
| 5 | 7 | 0 | -67 | -104 | -104 | -58 | -58 | -98 | -92 | -49 | -70 | -73 | -52 | -12 |
| 5 | 8 | 0 | -82 | -122 | -119 | -79 | -70 | -116 | -116 | -46 | -91 | -64 | -61 | 16 |
| 5 | 9 | 0 | -103 | -116 | -116 | -88 | -100 | -137 | -128 | -94 | -113 | -76 | -76 | -15 |
| 5 | 10 | 0 | -100 | -116 | -113 | -67 | -82 | -110 | -82 | -58 | -79 | -49 | -67 | 6 |
| 5 | 11 | 0 | -43 | -86 | -101 | -43 | -58 | -95 | -80 | -55 | -86 | -58 | -43 | 9 |
| 5 | 12 | 0 | -64 | -98 | -101 | -49 | -61 | -107 | -92 | -55 | -95 | -52 | -46 | 9 |
| 5 | 13 | 0 | -82 | -116 | -125 | -58 | -79 | -113 | -104 | -82 | -79 | -67 | -64 | -15 |
| 5 | 14 | 0 | -91 | -122 | -137 | -73 | -95 | -119 | -128 | -64 | -91 | -76 | -76 | -12 |
| 5 | 15 | 0 | -64 | -98 | -128 | -70 | -61 | -125 | -86 | -67 | -101 | -58 | -52 | 3 |
| 5 | 16 | 0 | -73 | -107 | -107 | -76 | -79 | -122 | -113 | -76 | -104 | -76 | -73 | 6 |
| 5 | 17 | 0 | -73 | -104 | -85 | -70 | -79 | -113 | -113 | -43 | -104 | -61 | -79 | -18 |
| 5 | 18 | 0 | -82 | -110 | -110 | -67 | -79 | -122 | -104 | -79 | -104 | -55 | -91 | -6 |
| 5 | 19 | 0 | -82 | -98 | -122 | -73 | -85 | -104 | -101 | -58 | -92 | -73 | -67 | -3 |
| 5 | 20 | 0 | -88 | -104 | -107 | -85 | -98 | -107 | -113 | -61 | -98 | -76 | -61 | 3 |
| 5 | 21 | 0 | -106 | -140 | -143 | -97 | -113 | -143 | -131 | -97 | -106 | -79 | -88 | -21 |
| 5 | 22 | 0 | -94 | -131 | -155 | -97 | -94 | -134 | -131 | -70 | -113 | -88 | -94 | -15 |
| 5 | 23 | 0 | -73 | -104 | -104 | -73 | -82 | -122 | -113 | -61 | -85 | -70 | -52 | 6 |
| 5 | 24 | 0 | -85 | -125 | -122 | -85 | -79 | -137 | -128 | -70 | -106 | -67 | -85 | 19 |
| 5 | 25 | 0 | -100 | -137 | -131 | -97 | -125 | -149 | -143 | -116 | -143 | -107 | -122 | -36 |
| 5 | 26 | 0 | -85 | -134 | -134 | -91 | -107 | -152 | -122 | -82 | -122 | -91 | -97 | -9 |
| 5 | 27 | 0 | -122 | -153 | -159 | -132 | -110 | -144 | -150 | -101 | -144 | -107 | -104 | -10 |
| 5 | 28 | 0 | -110 | -147 | -147 | -92 | -104 | -113 | -46 | 570 | 2423 | 6021 | 12623 | 30965 |
| 5 | 29 | 0 | -125 | -165 | -171 | -129 | -132 | -150 | -156 | -113 | -144 | -104 | -107 | -31 |
| 5 | 30 | 0 | -76 | -92 | -107 | -73 | -79 | -116 | -104 | -64 | -110 | -86 | -70 | 27 |
| 5 | 31 | 0 | -116 | -128 | -159 | -107 | -94 | -88 | 147 | 1248 | 3943 | 8360 | 16933 | 31820 |
| 5 | 32 | 0 | -76 | -110 | -131 | -88 | -82 | -128 | -131 | -49 | -101 | -79 | -70 | 34 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*K. pneumoniae* FRHKPC2 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -103 | -195 | -146 | -152 | -128 | -15 | 403 | 2439 | 6358 | 10557 | 17012 | 32922 |
| 20 | 2 | 0 | -97 | -192 | -164 | -146 | -134 | -51 | 397 | 2549 | 6797 | 11058 | 18050 | 32864 |
| 20 | 3 | 0 | -52 | -177 | -110 | -101 | -92 | 155 | 921 | 4236 | 11146 | 17906 | 24910 | 33560 |
| 20 | 4 | 0 | -64 | -174 | -125 | -122 | -107 | -12 | 290 | 1734 | 5243 | 9995 | 17732 | 32617 |
| 20 | 5 | 0 | -91 | -180 | -134 | -91 | -131 | -113 | -107 | 138 | 278 | 516 | 1780 | 31042 |
| 20 | 6 | 0 | -86 | -162 | -125 | -113 | -141 | -144 | -196 | -95 | -40 | 21 | 1538 | 29919 |
| 20 | 7 | 0 | -64 | -186 | -116 | -140 | -137 | -140 | -189 | 156 | 1102 | 3244 | 8689 | 29391 |
| 20 | 8 | 0 | -91 | -210 | -155 | -165 | -168 | -195 | -238 | -143 | -107 | 52 | 714 | 24871 |
| 20 | 9 | 0 | -112 | -180 | -137 | -137 | -106 | 116 | 864 | 3989 | 8934 | 13191 | 19518 | 33521 |
| 20 | 10 | 0 | -104 | -193 | -159 | -123 | -123 | -34 | 390 | 2643 | 7254 | 12037 | 19581 | 33489 |
| 20 | 11 | 0 | -58 | -153 | -116 | -113 | -107 | -95 | 12 | 1105 | 4178 | 8591 | 17048 | 33602 |
| 20 | 12 | 0 | -85 | -180 | -143 | -113 | -122 | -27 | 327 | 2091 | 6116 | 10890 | 18160 | 32736 |
| 20 | 13 | 0 | -82 | -159 | -113 | -110 | -107 | -43 | 226 | 1920 | 5198 | 9357 | 16466 | 31716 |
| 20 | 14 | 0 | -48 | -149 | -94 | -88 | -94 | -58 | -21 | 364 | 1529 | 4209 | 10380 | 30905 |
| 20 | 15 | 0 | -71 | -147 | -101 | -110 | -113 | -110 | -141 | 39 | 509 | 2020 | 6500 | 30221 |
| 20 | 16 | 0 | -86 | -168 | -131 | -125 | -137 | -125 | -180 | -89 | -122 | -147 | 6 | 28338 |
| 20 | 17 | 0 | -109 | -219 | -140 | -140 | -140 | -143 | -113 | 577 | 2097 | 4380 | 10225 | 33786 |
| 20 | 18 | 0 | -97 | -183 | -161 | -134 | -119 | -61 | 379 | 3153 | 7945 | 13127 | 21117 | 33383 |
| 20 | 19 | 0 | -46 | -177 | -98 | -86 | 30 | 454 | 1672 | 5911 | 12254 | 17289 | 23442 | 33346 |
| 20 | 20 | 0 | -64 | -141 | -101 | -89 | -74 | 100 | 537 | 2417 | 6366 | 11182 | 18828 | 33379 |
| 20 | 21 | 0 | -64 | -159 | -110 | -98 | -131 | -101 | -74 | 522 | 1782 | 4028 | 9833 | 32131 |
| 20 | 22 | 0 | -43 | -141 | -98 | -80 | -104 | -95 | -181 | 3 | 219 | 766 | 2658 | 31667 |
| 20 | 23 | 0 | -55 | -155 | -91 | -82 | -100 | -94 | -103 | 232 | 705 | 1905 | 6400 | 30801 |
| 20 | 24 | 0 | -88 | -171 | -143 | -119 | -110 | -113 | -128 | 217 | 1105 | 3324 | 8866 | 30004 |
| 20 | 25 | 0 | -97 | -174 | -134 | -128 | -113 | -21 | 318 | 2091 | 5430 | 9584 | 17003 | 33847 |
| 20 | 26 | 0 | -95 | -186 | -131 | -119 | -101 | 110 | 888 | 4071 | 9531 | 14488 | 21395 | 33151 |
| 20 | 27 | 0 | -107 | -189 | -140 | -131 | -125 | -104 | 86 | 1401 | 4420 | 8518 | 16472 | 32742 |
| 20 | 28 | 0 | -85 | -183 | -131 | -116 | -128 | -128 | -143 | 186 | 803 | 1932 | 5417 | 32418 |
| 20 | 29 | 0 | -39 | -140 | -67 | -61 | -27 | 165 | 586 | 2741 | 7182 | 12330 | 20134 | 32306 |
| 20 | 30 | 0 | -80 | -162 | -110 | -101 | -95 | -43 | 186 | 1397 | 3964 | 7874 | 15492 | 33022 |
| 20 | 31 | 0 | -73 | -177 | -104 | -125 | -128 | -119 | -113 | 488 | 1697 | 3586 | 9025 | 31265 |
| 20 | 32 | 0 | -67 | -171 | -116 | -107 | -119 | -113 | -101 | 174 | 1004 | 3192 | 8579 | 30547 |
| 10 | 1 | 0 | -106 | -201 | -152 | -143 | -146 | -91 | 190 | 2271 | 5940 | 10292 | 18083 | 33771 |
| 10 | 2 | 0 | -128 | -189 | -140 | -131 | -137 | -155 | -204 | -122 | -125 | -140 | -88 | 19 |
| 10 | 3 | 0 | -92 | -208 | -150 | -131 | -113 | 37 | 424 | 1935 | 4822 | 8793 | 16667 | 32119 |
| 10 | 4 | 0 | -70 | -162 | -135 | -101 | -77 | 21 | 326 | 1813 | 5017 | 9089 | 15699 | 32519 |
| 10 | 5 | 0 | -89 | -186 | -137 | -107 | -131 | -107 | -119 | 507 | 2310 | 5500 | 12568 | 32015 |
| 10 | 6 | 0 | -49 | -143 | -101 | -79 | -98 | -88 | -79 | 562 | 2115 | 4737 | 11161 | 31896 |
| 10 | 7 | 0 | -52 | -165 | -101 | -104 | -110 | -113 | -171 | -74 | -16 | 262 | 1693 | 31093 |
| 10 | 8 | 0 | -58 | -168 | -107 | -110 | -135 | -107 | -52 | 699 | 2551 | 5075 | 11115 | 31475 |
| 10 | 9 | 0 | -106 | -186 | -155 | -149 | -146 | -158 | -204 | 107 | 870 | 2399 | 7017 | 33597 |
| 10 | 10 | 0 | -89 | -165 | -119 | -116 | -95 | -34 | 214 | 1675 | 4895 | 8564 | 15748 | 32656 |
| 10 | 11 | 0 | -86 | -177 | -119 | -98 | -128 | -122 | -144 | 229 | 1254 | 3293 | 8588 | 32412 |
| 10 | 12 | 0 | -83 | -168 | -122 | -101 | -119 | -86 | 174 | 2008 | 5637 | 10206 | 17909 | 32403 |
| 10 | 13 | 0 | -86 | -147 | -116 | -116 | -132 | -89 | 12 | 1074 | 3839 | 7453 | 15119 | 31893 |
| 10 | 14 | 0 | -67 | -174 | -94 | -100 | -100 | -27 | 80 | 724 | 1453 | 2259 | 4453 | 31558 |
| 10 | 15 | 0 | -83 | -150 | -107 | -119 | -119 | -89 | 24 | 958 | 3622 | 7089 | 14417 | 31866 |
| 10 | 16 | 0 | -71 | -162 | -119 | -110 | -132 | -83 | 36 | 1126 | 3534 | 7092 | 13825 | 31771 |
| 10 | 17 | 0 | -122 | -195 | -164 | -170 | -161 | -177 | -201 | 382 | 1850 | 4527 | 9990 | 33545 |
| 10 | 18 | 0 | -98 | -177 | -146 | -134 | -137 | -153 | -189 | -101 | -113 | -149 | -82 | 31 |
| 10 | 19 | 0 | -107 | -186 | -119 | -128 | -140 | -131 | 30 | 1370 | 4554 | 8790 | 16493 | 32147 |
| 10 | 20 | 0 | -107 | -171 | -122 | -107 | -92 | 30 | 342 | 1758 | 4547 | 8295 | 15672 | 32363 |
| 10 | 21 | 0 | -80 | -141 | -107 | -104 | -110 | -110 | -174 | -74 | -89 | -125 | -61 | 48 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*K. pneumoniae* FRHKPC2 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -61 | -170 | -119 | -100 | -94 | 22 | 446 | 2747 | 7295 | 11781 | 18532 | 32315 |
| 10 | 23 | 0 | -68 | -153 | -116 | -113 | -95 | 0 | 152 | 973 | 2804 | 5334 | 11460 | 31896 |
| 10 | 24 | 0 | -68 | -156 | -104 | -110 | -89 | -119 | -174 | -77 | -101 | -135 | -55 | 33 |
| 10 | 25 | 0 | -116 | -198 | -161 | -128 | -70 | 138 | 495 | 1603 | 2634 | 3461 | 5299 | 32800 |
| 10 | 26 | 0 | -104 | -189 | -143 | -143 | -152 | -158 | -122 | 611 | 2686 | 5711 | 12587 | 32394 |
| 10 | 27 | 0 | -98 | -177 | -141 | -104 | -89 | 52 | 372 | 1743 | 4395 | 7703 | 14063 | 31588 |
| 10 | 28 | 0 | -80 | -162 | -116 | -107 | -125 | -116 | -183 | -70 | -116 | -144 | -67 | 42 |
| 10 | 29 | 0 | -52 | -165 | -116 | -119 | -122 | -104 | 52 | 1572 | 5145 | 9321 | 16655 | 31805 |
| 10 | 30 | 0 | -91 | -177 | -119 | -122 | -131 | -94 | -79 | 748 | 3062 | 6404 | 13359 | 32129 |
| 10 | 31 | 0 | -86 | -168 | -119 | -138 | -104 | -125 | -92 | 613 | 2969 | 6000 | 12507 | 31915 |
| 10 | 32 | 0 | -68 | -147 | -98 | -107 | -104 | -16 | 518 | 3329 | 8338 | 13410 | 20323 | 32983 |
| 5 | 1 | 0 | -98 | -183 | -149 | -146 | -146 | -143 | -211 | -116 | -116 | -156 | -88 | 24 |
| 5 | 2 | 0 | -82 | -180 | -122 | -107 | -125 | -128 | -183 | -95 | -113 | -162 | -95 | 15 |
| 5 | 3 | 0 | -98 | -186 | -128 | -110 | -137 | -85 | 46 | 632 | 1377 | 2072 | 3357 | 30441 |
| 5 | 4 | 0 | -110 | -213 | -149 | -146 | -158 | -143 | -192 | 205 | 1133 | 2906 | 8064 | 32095 |
| 5 | 5 | 0 | -74 | -177 | -125 | -113 | -125 | -89 | 52 | 1236 | 4041 | 7679 | 14201 | 32134 |
| 5 | 6 | 0 | -74 | -165 | -113 | -98 | -55 | 103 | 418 | 1703 | 4147 | 7404 | 14448 | 32333 |
| 5 | 7 | 0 | -71 | -165 | -116 | -113 | -113 | -95 | 70 | 1455 | 4651 | 8759 | 15403 | 32101 |
| 5 | 8 | 0 | -89 | -147 | -128 | -119 | -122 | -119 | -89 | 821 | 3363 | 6586 | 13511 | 32687 |
| 5 | 9 | 0 | -92 | -171 | -137 | -131 | -122 | 0 | 522 | 3220 | 7716 | 12043 | 18175 | 33496 |
| 5 | 10 | 0 | -95 | -186 | -131 | -131 | -128 | -131 | -186 | -92 | -107 | -153 | -64 | 28 |
| 5 | 11 | 0 | -83 | -180 | -125 | -131 | -141 | -113 | -34 | 1025 | 3757 | 7441 | 14765 | 32446 |
| 5 | 12 | 0 | -98 | -192 | -128 | -128 | -95 | 6 | 98 | 699 | 1496 | 2668 | 6467 | 32260 |
| 5 | 13 | 0 | -74 | -177 | -159 | -119 | -113 | -68 | 119 | 1413 | 4303 | 8286 | 15794 | 32482 |
| 5 | 14 | 0 | -101 | -177 | -119 | -128 | -150 | -37 | 409 | 2905 | 7163 | 12043 | 19151 | 32641 |
| 5 | 15 | 0 | -62 | -147 | -104 | -116 | -80 | 9 | 503 | 2954 | 7080 | 11390 | 17610 | 32363 |
| 5 | 16 | 0 | -95 | -174 | -122 | -119 | -125 | -55 | 201 | 1749 | 4828 | 9132 | 15983 | 33016 |
| 5 | 17 | 0 | -125 | -189 | -152 | -164 | -161 | -158 | -225 | -125 | -106 | -189 | -100 | 25 |
| 5 | 18 | 0 | -67 | -168 | -143 | -125 | -104 | 79 | 705 | 3662 | 8787 | 13520 | 19722 | 33062 |
| 5 | 19 | 0 | -98 | -177 | -143 | -143 | -143 | -101 | -6 | 1325 | 4370 | 8912 | 16633 | 32437 |
| 5 | 20 | 0 | -77 | -186 | -122 | -113 | -138 | -131 | -177 | -95 | -104 | -138 | -61 | 52 |
| 5 | 21 | 0 | -97 | -180 | -131 | -125 | -122 | -113 | 6 | 901 | 2949 | 6461 | 12706 | 32675 |
| 5 | 22 | 0 | -73 | -168 | -107 | -119 | -110 | -113 | -61 | 797 | 3071 | 6684 | 13087 | 32052 |
| 5 | 23 | 0 | -70 | -192 | -106 | -103 | -91 | -119 | -158 | -76 | -67 | -128 | -54 | 83 |
| 5 | 24 | 0 | -80 | -162 | -104 | -110 | -110 | 6 | 442 | 2396 | 6082 | 10572 | 17189 | 33050 |
| 5 | 25 | 0 | -125 | -226 | -183 | -180 | -180 | -165 | -210 | 83 | 370 | 699 | 1493 | 32944 |
| 5 | 26 | 0 | -116 | -217 | -134 | -137 | -162 | -146 | -134 | 485 | 2259 | 5021 | 11265 | 34387 |
| 5 | 27 | 0 | -80 | -177 | -147 | -134 | -137 | -61 | 165 | 1501 | 4581 | 8561 | 14854 | 34558 |
| 5 | 28 | 0 | -76 | -164 | -112 | -97 | -100 | -36 | 95 | 934 | 2772 | 5793 | 11772 | 34540 |
| 5 | 29 | 0 | -79 | -171 | -141 | -137 | -125 | -79 | 122 | 1190 | 3937 | 8191 | 14683 | 34848 |
| 5 | 30 | 0 | -110 | -183 | -155 | -143 | -162 | -152 | -210 | -122 | -134 | -171 | -94 | 12 |
| 5 | 31 | 0 | -68 | -153 | -129 | -110 | -135 | -129 | -86 | 790 | 2957 | 6622 | 13193 | 33190 |
| 5 | 32 | 0 | -71 | -174 | -132 | -116 | -135 | -116 | -28 | 851 | 3076 | 6952 | 13941 | 33944 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. cloacae* FRHECL2 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -223 | -217 | -266 | -135 | -238 | -119 | -16 | 476 | 1135 | 2499 | 6848 | 36236 |
| 20 | 2 | 0 | -232 | -214 | -290 | -144 | -244 | -101 | -22 | 378 | 845 | 1816 | 4398 | 37210 |
| 20 | 3 | 0 | -208 | -202 | -266 | -129 | -208 | -77 | 33 | 467 | 952 | 2078 | 5106 | 37921 |
| 20 | 4 | 0 | -208 | -192 | -257 | -144 | -217 | -131 | -135 | 158 | 433 | 1166 | 3119 | 38333 |
| 20 | 5 | 0 | -199 | -196 | -260 | -132 | -226 | -104 | -13 | 418 | 930 | 2243 | 5850 | 39190 |
| 20 | 6 | 0 | -217 | -205 | -254 | -162 | -186 | 73 | 351 | 1578 | 3665 | 7798 | 15495 | 39032 |
| 20 | 7 | 0 | -158 | -186 | -213 | -128 | -183 | 19 | 220 | 1120 | 2887 | 7127 | 15169 | 39450 |
| 20 | 8 | 0 | -223 | -199 | -260 | -147 | -199 | 6 | 207 | 1071 | 2420 | 6055 | 13285 | 38629 |
| 20 | 9 | 0 | -214 | -196 | -257 | -132 | -205 | -46 | 79 | 573 | 1147 | 2276 | 5466 | 38195 |
| 20 | 10 | 0 | -196 | -180 | -254 | -132 | -199 | -25 | 116 | 723 | 1513 | 3320 | 8219 | 38784 |
| 20 | 11 | 0 | -183 | -165 | -229 | -104 | -204 | -119 | -95 | 241 | 574 | 1703 | 5439 | 38657 |
| 20 | 12 | 0 | -220 | -208 | -272 | -159 | -208 | 12 | 265 | 1508 | 3198 | 7343 | 13886 | 38989 |
| 20 | 13 | 0 | -189 | -171 | -241 | -128 | -201 | -64 | 67 | 663 | 1593 | 4771 | 10521 | 38904 |
| 20 | 14 | 0 | -202 | -184 | -269 | -150 | -190 | -16 | 207 | 1220 | 3073 | 7166 | 13734 | 38897 |
| 20 | 15 | 0 | -173 | -186 | -253 | -125 | -167 | 119 | 452 | 2323 | 5399 | 10402 | 18306 | 39481 |
| 20 | 16 | 0 | -208 | -193 | -254 | -150 | -141 | 180 | 582 | 2453 | 5872 | 11179 | 19889 | 39926 |
| 20 | 17 | 0 | -220 | -205 | -260 | -113 | -186 | 52 | 284 | 1355 | 3122 | 6565 | 13276 | 39145 |
| 20 | 18 | 0 | -216 | -183 | -262 | -140 | -192 | -97 | -15 | 458 | 1060 | 3022 | 8207 | 38816 |
| 20 | 19 | 0 | -189 | -159 | -235 | -125 | -177 | 37 | 278 | 1383 | 3327 | 7114 | 13642 | 38681 |
| 20 | 20 | 0 | -189 | -177 | -256 | -113 | -131 | 193 | 599 | 2802 | 5143 | 9443 | 15944 | 38626 |
| 20 | 21 | 0 | -186 | -195 | -253 | -140 | -180 | 10 | 263 | 1294 | 3303 | 7099 | 13274 | 38275 |
| 20 | 22 | 0 | -214 | -184 | -254 | -135 | -171 | 48 | 344 | 1751 | 4739 | 8960 | 15818 | 38839 |
| 20 | 23 | 0 | -223 | -232 | -259 | -177 | -207 | -24 | 226 | 1502 | 3882 | 8186 | 15053 | 40015 |
| 20 | 24 | 0 | -214 | -190 | -248 | -159 | -180 | 33 | 280 | 1318 | 3717 | 8408 | 16664 | 39785 |
| 20 | 25 | 0 | -183 | -186 | -241 | -125 | -204 | -91 | 3 | 519 | 1206 | 3712 | 8921 | 39304 |
| 20 | 26 | 0 | -180 | -180 | -226 | -113 | -183 | 12 | 223 | 1004 | 2201 | 5701 | 11879 | 38706 |
| 20 | 27 | 0 | -186 | -177 | -223 | -101 | -162 | 37 | 263 | 1261 | 3064 | 6772 | 12550 | 38309 |
| 20 | 28 | 0 | -196 | -181 | -257 | -144 | -193 | 9 | 235 | 1275 | 3534 | 6885 | 12992 | 38314 |
| 20 | 29 | 0 | -165 | -165 | -232 | -107 | -162 | 98 | 394 | 1944 | 5051 | 8521 | 16316 | 38153 |
| 20 | 30 | 0 | -189 | -189 | -226 | -119 | -153 | 122 | 436 | 2152 | 5591 | 10206 | 17775 | 38434 |
| 20 | 31 | 0 | -174 | -168 | -220 | -98 | -98 | 293 | 891 | 4346 | 8772 | 14073 | 22463 | 38525 |
| 20 | 32 | 0 | -198 | -192 | -250 | -143 | -186 | 80 | 379 | 1972 | 4545 | 9111 | 16179 | 39466 |
| 10 | 1 | 0 | -223 | -202 | -263 | -150 | -229 | -107 | 15 | 558 | 1376 | 4309 | 10444 | 39102 |
| 10 | 2 | 0 | -201 | -186 | -259 | -143 | -222 | -119 | -51 | 394 | 876 | 3080 | 8100 | 38425 |
| 10 | 3 | 0 | -174 | -156 | -226 | -107 | -174 | -95 | 15 | 418 | 964 | 3097 | 8432 | 37655 |
| 10 | 4 | 0 | -180 | -171 | -235 | -134 | -195 | -82 | -3 | 504 | 1276 | 3632 | 9410 | 37790 |
| 10 | 5 | 0 | -205 | -177 | -232 | -92 | -177 | -64 | 24 | 571 | 1459 | 4523 | 11369 | 37536 |
| 10 | 6 | 0 | -198 | -171 | -205 | -128 | -208 | 0 | 149 | 925 | 2463 | 6403 | 13883 | 37762 |
| 10 | 7 | 0 | -165 | -168 | -229 | -83 | -171 | 9 | 146 | 940 | 2536 | 6208 | 13630 | 38046 |
| 10 | 8 | 0 | -198 | -177 | -247 | -131 | -201 | -51 | 65 | 879 | 2250 | 5735 | 11561 | 38627 |
| 10 | 9 | 0 | -207 | -204 | -253 | -140 | -225 | -131 | -91 | 315 | 834 | 2582 | 8345 | 39002 |
| 10 | 10 | 0 | -198 | -195 | -253 | -140 | -216 | -164 | -219 | -91 | 13 | 422 | 1648 | 38199 |
| 10 | 11 | 0 | -177 | -198 | -238 | -125 | -211 | -79 | 52 | 528 | 1202 | 3845 | 10462 | 37567 |
| 10 | 12 | 0 | -171 | -180 | -235 | -116 | -192 | -122 | -177 | 34 | 226 | 812 | 3317 | 37323 |
| 10 | 13 | 0 | -186 | -165 | -226 | -122 | -199 | -128 | -128 | 110 | 372 | 1230 | 4654 | 37085 |
| 10 | 14 | 0 | -177 | -174 | -217 | -131 | -195 | -95 | -64 | 360 | 912 | 2835 | 8649 | 37497 |
| 10 | 15 | 0 | -168 | -159 | -214 | -104 | -162 | 58 | 509 | 2832 | 6638 | 11973 | 19825 | 37283 |
| 10 | 16 | 0 | -189 | -159 | -226 | -137 | -195 | -152 | -241 | -186 | -247 | -220 | -110 | 12 |
| 10 | 17 | 0 | -214 | -196 | -254 | -126 | -229 | -159 | -165 | 128 | 531 | 1516 | 6009 | 38803 |
| 10 | 18 | 0 | -195 | -189 | -265 | -137 | -210 | -171 | -235 | -164 | -164 | 64 | 693 | 37949 |
| 10 | 19 | 0 | -189 | -192 | -232 | -125 | -195 | -79 | 43 | 583 | 1425 | 4599 | 11405 | 37723 |
| 10 | 20 | 0 | -189 | -189 | -253 | -131 | -226 | -131 | -119 | 202 | 583 | 1865 | 6754 | 37567 |
| 10 | 21 | 0 | -198 | -165 | -226 | -119 | -211 | -122 | -107 | 244 | 729 | 2225 | 7856 | 37433 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. cloacae* FRHECL2 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -180 | -174 | -232 | -113 | -177 | -25 | 174 | 1108 | 3085 | 6980 | 14036 | 37805 |
| 10 | 23 | 0 | -201 | -180 | -256 | -134 | -204 | -107 | -46 | 443 | 1380 | 3952 | 9531 | 37631 |
| 10 | 24 | 0 | -189 | -174 | -238 | -140 | -201 | -79 | 12 | 546 | 1471 | 4172 | 10105 | 37949 |
| 10 | 25 | 0 | -205 | -202 | -272 | -144 | -211 | -123 | -116 | 283 | 769 | 2142 | 7083 | 38751 |
| 10 | 26 | 0 | -165 | -189 | -241 | -143 | -195 | -82 | 0 | 516 | 1245 | 3980 | 10865 | 38104 |
| 10 | 27 | 0 | -189 | -171 | -244 | -128 | -198 | -113 | -119 | 201 | 561 | 1700 | 6415 | 36813 |
| 10 | 28 | 0 | -171 | -168 | -238 | -125 | -198 | -98 | -18 | 470 | 1172 | 3958 | 10151 | 37402 |
| 10 | 29 | 0 | -189 | -171 | -214 | -134 | -192 | -113 | -125 | 204 | 598 | 1962 | 6833 | 36831 |
| 10 | 30 | 0 | -174 | -159 | -217 | -129 | -190 | -113 | -89 | 296 | 821 | 2862 | 7785 | 37295 |
| 10 | 31 | 0 | -183 | -171 | -226 | -107 | -183 | -89 | -12 | 491 | 1599 | 4315 | 9995 | 37384 |
| 10 | 32 | 0 | -207 | -168 | -220 | -128 | -204 | -110 | -88 | 260 | 693 | 2506 | 7505 | 38284 |
| 5 | 1 | 0 | -186 | -180 | -247 | -125 | -183 | -91 | -24 | 430 | 1154 | 3324 | 9199 | 38556 |
| 5 | 2 | 0 | -207 | -183 | -247 | -134 | -213 | -158 | -180 | 31 | 269 | 879 | 3409 | 37827 |
| 5 | 3 | 0 | -210 | -189 | -241 | -140 | -216 | -168 | -186 | 49 | 254 | 864 | 3385 | 36948 |
| 5 | 4 | 0 | -174 | -201 | -262 | -143 | -220 | -180 | -226 | -195 | -278 | -268 | -140 | 9 |
| 5 | 5 | 0 | -171 | -162 | -217 | -125 | -205 | -141 | -238 | -150 | -257 | -238 | -119 | -9 |
| 5 | 6 | 0 | -162 | -162 | -244 | -141 | -180 | -131 | -159 | -52 | -28 | 158 | 784 | 35940 |
| 5 | 7 | 0 | -177 | -171 | -223 | -107 | -186 | -137 | -223 | -180 | -250 | -220 | -119 | 6 |
| 5 | 8 | 0 | -189 | -174 | -256 | -155 | -198 | -88 | -88 | 263 | 617 | 2109 | 6388 | 37595 |
| 5 | 9 | 0 | -198 | -177 | -241 | -131 | -216 | -152 | -210 | -79 | 52 | 385 | 1578 | 38593 |
| 5 | 10 | 0 | -198 | -186 | -241 | -125 | -207 | -140 | -168 | 3 | 196 | 721 | 2601 | 37949 |
| 5 | 11 | 0 | -201 | -174 | -235 | -116 | -201 | -119 | -119 | 229 | 568 | 1734 | 6608 | 37161 |
| 5 | 12 | 0 | -158 | -168 | -250 | -128 | -216 | -155 | -216 | -104 | -6 | 339 | 1520 | 36649 |
| 5 | 13 | 0 | -204 | -180 | -259 | -161 | -229 | -149 | -250 | -198 | -277 | -262 | -149 | -6 |
| 5 | 14 | 0 | -183 | -168 | -241 | -140 | -204 | -153 | -204 | -73 | 9 | 394 | 1776 | 36560 |
| 5 | 15 | 0 | -177 | -156 | -226 | -95 | -211 | -153 | -226 | -177 | -263 | -214 | -107 | 9 |
| 5 | 16 | 0 | -201 | -180 | -235 | -143 | -216 | -113 | -134 | 119 | 376 | 1355 | 5079 | 37598 |
| 5 | 17 | 0 | -195 | -174 | -247 | -134 | -232 | -167 | -241 | -143 | -79 | 165 | 992 | 38770 |
| 5 | 18 | 0 | -216 | -192 | -256 | -140 | -238 | -173 | -250 | -146 | -58 | 235 | 1200 | 38367 |
| 5 | 19 | 0 | -204 | -189 | -238 | -134 | -214 | -119 | -104 | 290 | 794 | 2912 | 9016 | 37824 |
| 5 | 20 | 0 | -198 | -195 | -259 | -158 | -232 | -164 | -232 | -140 | -198 | -110 | 187 | 37210 |
| 5 | 21 | 0 | -204 | -171 | -244 | -159 | -217 | -134 | -159 | 128 | 455 | 1453 | 5436 | 37265 |
| 5 | 22 | 0 | -186 | -165 | -220 | -125 | -190 | -144 | -177 | -22 | 125 | 662 | 2728 | 37359 |
| 5 | 23 | 0 | -174 | -165 | -217 | -122 | -189 | -116 | -119 | 168 | 620 | 2683 | 7996 | 37973 |
| 5 | 24 | 0 | -210 | -192 | -262 | -155 | -219 | -152 | -195 | -21 | 138 | 626 | 2180 | 37711 |
| 5 | 25 | 0 | -260 | -223 | -272 | -180 | -232 | -25 | 170 | 1272 | 3226 | 6323 | 13792 | 39557 |
| 5 | 26 | 0 | -238 | -195 | -271 | -137 | -213 | -73 | 86 | 928 | 2918 | 5811 | 12858 | 40009 |
| 5 | 27 | 0 | -213 | -207 | -283 | -158 | -232 | -140 | -122 | 217 | 611 | 1951 | 6468 | 39496 |
| 5 | 28 | 0 | -223 | -192 | -262 | -137 | -213 | -159 | -171 | 110 | 385 | 1337 | 4529 | 39774 |
| 5 | 29 | 0 | -226 | -214 | -260 | -156 | -235 | -165 | -235 | -58 | 61 | 500 | 2252 | 39389 |
| 5 | 30 | 0 | -192 | -186 | -250 | -131 | -201 | -146 | -168 | 43 | 226 | 827 | 3107 | 39536 |
| 5 | 31 | 0 | -186 | -183 | -235 | -116 | -205 | -150 | -177 | 73 | 320 | 1196 | 4251 | 39169 |
| 5 | 32 | 0 | -238 | -205 | -284 | -165 | -232 | -171 | -199 | 15 | 247 | 876 | 2603 | 38171 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. aerogenes* FRHEAE2 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 0 | -183 | -216 | -268 | -167 | -195 | -213 | -225 | 217 | -119 | 101 | 797 | 42106 |
| 20 | 2 | 0 | -226 | -271 | -302 | -204 | -241 | -238 | -259 | 232 | -98 | 226 | 1508 | 38303 |
| 20 | 3 | 0 | -219 | -235 | -277 | -180 | -204 | -201 | -229 | 159 | -164 | 10 | 641 | 39921 |
| 20 | 4 | 0 | -242 | -220 | -269 | -196 | -214 | -211 | -232 | 140 | -208 | -37 | 445 | 38458 |
| 20 | 5 | 0 | -219 | -241 | -247 | -177 | -213 | -195 | -238 | 202 | -189 | 6 | 693 | 37006 |
| 20 | 6 | 0 | -201 | -259 | -296 | -216 | -265 | -244 | -262 | 113 | -259 | -125 | 260 | 36930 |
| 20 | 7 | 0 | -183 | -241 | -247 | -149 | -217 | -229 | -229 | 174 | -128 | 144 | 1016 | 34161 |
| 20 | 8 | 0 | -214 | -232 | -275 | -171 | -211 | -214 | -226 | 287 | 18 | 482 | 1544 | 31539 |
| 20 | 9 | 0 | -140 | -159 | -201 | -101 | -131 | -140 | -150 | 403 | 49 | 412 | 1621 | 36929 |
| 20 | 10 | 0 | -143 | -186 | -225 | -103 | -161 | -158 | -210 | 229 | -186 | -61 | 290 | 35065 |
| 20 | 11 | 0 | -162 | -174 | -214 | -104 | -149 | -137 | -140 | 287 | -82 | 134 | 906 | 36630 |
| 20 | 12 | 0 | -155 | -168 | -213 | -119 | -140 | -152 | -162 | 229 | -174 | -67 | 275 | 34671 |
| 20 | 13 | 0 | -122 | -147 | -192 | -92 | -140 | -128 | -159 | 223 | -180 | -113 | 122 | 33905 |
| 20 | 14 | 0 | -104 | -165 | -205 | -119 | -138 | -135 | -156 | 247 | -171 | -37 | 314 | 35772 |
| 20 | 15 | 0 | -131 | -168 | -211 | -95 | -150 | -159 | -177 | 208 | -192 | -153 | 89 | 32852 |
| 20 | 16 | 0 | -162 | -174 | -226 | -137 | -171 | -146 | -189 | 192 | -192 | -110 | 250 | 31717 |
| 20 | 17 | 0 | -168 | -171 | -201 | -125 | -161 | -116 | -100 | 595 | 330 | 1169 | 3687 | 36606 |
| 20 | 18 | 0 | -158 | -186 | -235 | -113 | -152 | -158 | -164 | 357 | 25 | 281 | 1172 | 34573 |
| 20 | 19 | 0 | -153 | -165 | -189 | -113 | -119 | -144 | -134 | 281 | -107 | 43 | 510 | 33636 |
| 20 | 20 | 0 | -137 | -186 | -217 | -107 | -152 | -134 | -155 | 293 | -76 | 134 | 754 | 33978 |
| 20 | 21 | 0 | -107 | -156 | -205 | -101 | -150 | -131 | -150 | 329 | -31 | 177 | 793 | 33642 |
| 20 | 22 | 0 | -131 | -159 | -220 | -119 | -165 | -140 | -171 | 220 | -159 | 6 | 342 | 33801 |
| 20 | 23 | 0 | -143 | -168 | -217 | -107 | -174 | -159 | -177 | 226 | -140 | 12 | 427 | 33584 |
| 20 | 24 | 0 | -147 | -159 | -186 | -92 | -147 | -128 | -156 | 214 | -119 | 3 | 455 | 33117 |
| 20 | 25 | 0 | -174 | -207 | -219 | -116 | -155 | -137 | -143 | 415 | 71 | 425 | 1844 | 35724 |
| 20 | 26 | 0 | -156 | -159 | -217 | -92 | -122 | -140 | -162 | 275 | -101 | 37 | 491 | 34787 |
| 20 | 27 | 0 | -168 | -189 | -232 | -155 | -158 | -131 | -103 | 519 | 168 | 644 | 2250 | 34186 |
| 20 | 28 | 0 | -125 | -171 | -217 | -122 | -131 | -122 | -137 | 317 | -34 | 189 | 900 | 33560 |
| 20 | 29 | 0 | -116 | -159 | -187 | -80 | -119 | -98 | -37 | 683 | 405 | 1333 | 3741 | 34185 |
| 20 | 30 | 0 | -119 | -183 | -199 | -128 | -141 | -113 | -156 | 226 | -177 | -74 | 222 | 33114 |
| 20 | 31 | 0 | -129 | -147 | -193 | -83 | -135 | -129 | -153 | 253 | -144 | -31 | 329 | 32970 |
| 20 | 32 | 0 | -158 | -174 | -220 | -137 | -143 | -149 | -189 | 247 | -97 | 52 | 592 | 33597 |
| 10 | 1 | 0 | -153 | -165 | -204 | -113 | -134 | -140 | -174 | 272 | -107 | 31 | 449 | 35171 |
| 10 | 2 | 0 | -147 | -168 | -211 | -104 | -140 | -128 | -134 | 378 | 34 | 259 | 934 | 34256 |
| 10 | 3 | 0 | -150 | -147 | -211 | -132 | -132 | -113 | -126 | 369 | 36 | 253 | 1071 | 33327 |
| 10 | 4 | 0 | -119 | -168 | -199 | -98 | -122 | -113 | -113 | 400 | 52 | 317 | 1163 | 33813 |
| 10 | 5 | 0 | -165 | -156 | -217 | -110 | -146 | -134 | -183 | 226 | -201 | -92 | 208 | 32901 |
| 10 | 6 | 0 | -144 | -180 | -205 | -128 | -150 | -141 | -180 | 201 | -217 | -190 | -6 | 32738 |
| 10 | 7 | 0 | -135 | -153 | -202 | -104 | -141 | -135 | -153 | 305 | -52 | 146 | 699 | 32821 |
| 10 | 8 | 0 | -165 | -199 | -229 | -156 | -183 | -171 | -205 | 238 | -95 | 91 | 796 | 33984 |
| 10 | 9 | 0 | -149 | -177 | -223 | -116 | -140 | -125 | -122 | 473 | 147 | 479 | 1679 | 35382 |
| 10 | 10 | 0 | -174 | -177 | -239 | -159 | -177 | -153 | -147 | 442 | 106 | 549 | 1925 | 34295 |
| 10 | 11 | 0 | -141 | -150 | -189 | -134 | -156 | -141 | -162 | 217 | -180 | -104 | 152 | 33465 |
| 10 | 12 | 0 | -119 | -177 | -205 | -92 | -119 | -128 | -116 | 409 | 82 | 421 | 1620 | 34274 |
| 10 | 13 | 0 | -140 | -177 | -214 | -110 | -153 | -134 | -131 | 400 | 31 | 369 | 1612 | 33569 |
| 10 | 14 | 0 | -141 | -165 | -199 | -113 | -126 | -119 | -150 | 204 | -199 | -77 | 134 | 32567 |
| 10 | 15 | 0 | -153 | -171 | -214 | -144 | -150 | -141 | -141 | 262 | -116 | 12 | 476 | 32580 |
| 10 | 16 | 0 | -140 | -171 | -204 | -125 | -165 | -149 | -174 | 247 | -125 | 34 | 510 | 33862 |
| 10 | 17 | 0 | -161 | -177 | -235 | -113 | -143 | -168 | -198 | 226 | -161 | -85 | 266 | 33878 |
| 10 | 18 | 0 | -146 | -189 | -238 | -140 | -158 | -155 | -152 | 348 | -30 | 211 | 885 | 34247 |
| 10 | 19 | 0 | -165 | -180 | -208 | -137 | -156 | -147 | -134 | 354 | -3 | 207 | 1071 | 33966 |
| 10 | 20 | 0 | -101 | -180 | -211 | -95 | -131 | -131 | -104 | 390 | 61 | 320 | 1211 | 33929 |
| 10 | 21 | 0 | -162 | -165 | -214 | -107 | -138 | -147 | -159 | 195 | -192 | -141 | 101 | 32977 |

## Appendix 5.4 (Cont'd..): Fluorescence data of EHC-GAL and 4-MU-GAL over 24 h with 10 coliform organisms.

*E. aerogenes* FRHEAE2 with 4-MU-GAL

| Estimated inoculum | Replicate | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 22 | 0 | -129 | -153 | -187 | -119 | -129 | -144 | -150 | 207 | -205 | -119 | 119 | 32976 |
| 10 | 23 | 0 | -150 | -177 | -208 | -116 | -138 | -119 | -138 | 280 | -89 | 48 | 467 | 32674 |
| 10 | 24 | 0 | -140 | -177 | -204 | -119 | -165 | -149 | -168 | 202 | -204 | -137 | 134 | 33621 |
| 10 | 25 | 0 | -149 | -162 | -229 | -107 | -146 | -137 | -195 | 214 | -162 | -58 | 256 | 34161 |
| 10 | 26 | 0 | -146 | -153 | -192 | -134 | -146 | -143 | -156 | 287 | -116 | 37 | 421 | 33838 |
| 10 | 27 | 0 | -171 | -168 | -199 | -147 | -141 | -150 | -174 | 232 | -135 | -31 | 339 | 32729 |
| 10 | 28 | 0 | -101 | -168 | -217 | -101 | -122 | -128 | -119 | 430 | 97 | 357 | 1349 | 33770 |
| 10 | 29 | 0 | -159 | -159 | -205 | -116 | -156 | -113 | -135 | 357 | 18 | 235 | 1050 | 33065 |
| 10 | 30 | 0 | -146 | -167 | -189 | -119 | -134 | -161 | -146 | 245 | -109 | 65 | 507 | 32809 |
| 10 | 31 | 0 | -156 | -159 | -214 | -110 | -116 | -141 | -150 | 265 | -77 | 85 | 595 | 32854 |
| 10 | 32 | 0 | -134 | -150 | -201 | -125 | -146 | -131 | -140 | 336 | 3 | 223 | 815 | 34652 |
| 5 | 1 | 0 | -153 | -180 | -241 | -113 | -153 | -143 | -180 | 189 | -180 | -153 | 58 | 32434 |
| 5 | 2 | 0 | -141 | -168 | -211 | -119 | -131 | -141 | -153 | 275 | -107 | 46 | 427 | 33267 |
| 5 | 3 | 0 | -153 | -183 | -201 | -153 | -140 | -149 | -149 | 455 | 183 | 784 | 2863 | 33529 |
| 5 | 4 | 0 | -113 | -174 | -205 | -113 | -144 | -141 | -165 | 201 | -202 | -220 | -119 | 30 |
| 5 | 5 | 0 | -150 | -174 | -205 | -116 | -144 | -141 | -156 | 213 | -171 | -116 | 137 | 32006 |
| 5 | 6 | 0 | -147 | -153 | -208 | -116 | -138 | -138 | -138 | 213 | -220 | -208 | -110 | 45 |
| 5 | 7 | 0 | -134 | -158 | -183 | -82 | -106 | -128 | -137 | 235 | -167 | -76 | 226 | 32794 |
| 5 | 8 | 0 | -143 | -171 | -223 | -113 | -168 | -162 | -165 | 226 | -180 | -79 | 226 | 34118 |
| 5 | 9 | 0 | -137 | -162 | -220 | -104 | -159 | -150 | -153 | 287 | -34 | 137 | 589 | 33679 |
| 5 | 10 | 0 | -146 | -180 | -214 | -128 | -149 | -149 | -180 | 217 | -204 | -174 | 25 | 30941 |
| 5 | 11 | 0 | -155 | -198 | -220 | -122 | -143 | -149 | -165 | 257 | -143 | -55 | 223 | 31689 |
| 5 | 12 | 0 | -137 | -177 | -211 | -140 | -153 | -140 | -162 | 201 | -226 | -217 | -122 | 15 |
| 5 | 13 | 0 | -141 | -168 | -220 | -134 | -141 | -156 | -156 | 244 | -116 | -9 | 403 | 32617 |
| 5 | 14 | 0 | -168 | -180 | -217 | -140 | -150 | -156 | -159 | 238 | -165 | -31 | 342 | 32827 |
| 5 | 15 | 0 | -165 | -171 | -211 | -125 | -141 | -141 | -150 | 213 | -177 | -70 | 256 | 32314 |
| 5 | 16 | 0 | -167 | -180 | -229 | -134 | -152 | -161 | -189 | 193 | -238 | -201 | -3 | 34467 |
| 5 | 17 | 0 | -155 | -210 | -241 | -110 | -171 | -164 | -186 | 205 | -183 | -94 | 217 | 34293 |
| 5 | 18 | 0 | -147 | -168 | -211 | -125 | -137 | -150 | -156 | 302 | -61 | 217 | 1111 | 34207 |
| 5 | 19 | 0 | -153 | -177 | -229 | -122 | -150 | -162 | -177 | 207 | -183 | -113 | 146 | 33172 |
| 5 | 20 | 0 | -153 | -156 | -202 | -138 | -141 | -144 | -168 | 192 | -189 | -183 | -28 | 33135 |
| 5 | 21 | 0 | -159 | -183 | -214 | -156 | -159 | -156 | -174 | 189 | -229 | -238 | -140 | 15 |
| 5 | 22 | 0 | -144 | -180 | -211 | -122 | -159 | -129 | -122 | 345 | 0 | 265 | 1108 | 33117 |
| 5 | 23 | 0 | -165 | -180 | -214 | -122 | -147 | -153 | -156 | 226 | -140 | 0 | 363 | 33810 |
| 5 | 24 | 0 | -162 | -180 | -220 | -128 | -155 | -143 | -192 | 205 | -192 | -140 | 131 | 35327 |
| 5 | 25 | 0 | -159 | -195 | -250 | -125 | -189 | -186 | -208 | 171 | -232 | -214 | -49 | 35525 |
| 5 | 26 | 0 | -156 | -168 | -229 | -137 | -134 | -159 | -189 | 229 | -153 | -55 | 244 | 35382 |
| 5 | 27 | 0 | -192 | -183 | -238 | -137 | -171 | -177 | -204 | 153 | -244 | -247 | -52 | 34668 |
| 5 | 28 | 0 | -183 | -183 | -229 | -162 | -162 | -159 | -189 | 229 | -147 | -40 | 281 | 35855 |
| 5 | 29 | 0 | -168 | -180 | -214 | -144 | -165 | -177 | -171 | 262 | -116 | 27 | 479 | 35556 |
| 5 | 30 | 0 | -150 | -189 | -229 | -119 | -180 | -168 | -171 | 208 | -180 | -64 | 296 | 35107 |
| 5 | 31 | 0 | -144 | -186 | -229 | -116 | -135 | -156 | -171 | 229 | -183 | -89 | 164 | 36215 |
| 5 | 32 | 0 | -168 | -180 | -220 | -116 | -156 | -162 | -162 | 256 | -116 | -19 | 369 | 37491 |

## Appendix 5.5: Pattern of positive/negative wells of EHC-GAL, 4-MU-GAL and LTB with 10 coliform organisms.

*C. freundii* NCTC 9750 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 9 | 22 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 9 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 5 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.7 | 2.8 | 8.9 |

*K. pneumoniae* NCTC 10896 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 1 | 8 | 12 | 25 | 27 | 29 | 29 | 30 | 30 | 30 |
| 10 | 0 | 0 | 0 | 0 | 3 | 5 | 12 | 14 | 20 | 20 | 27 | 27 | 27 |
| 5 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 12 | 12 | 12 | 12 | 12 |
| MPN | 0 | 0 | 0 | 0.2 | 2.2 | 4 | 10.6 | 12.4 | 20.8 | 20.8 | 27.7 | 27.7 | 27.7 |

*E. cloacae* NCTC 11936 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 2 | 4 | 16 | 20 | 23 | 23 | 23 | 23 | 23 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 6 | 10 | 10 | 13 | 14 | 15 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 5 | 7 | 7 | 7 | 8 | 8 |
| MPN | 0 | 0 | 0 | 0 | 0.4 | 0.7 | 4.8 | 7.3 | 10.3 | 10.3 | 11.3 | 12 | 12.4 |

*E. coli* NCIMB 10213 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 0.2 | 0.2 | 0.4 | 0.4 |

*E. aerogenes* NCIMB 10102 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 16 | 26 | 32 | 32 | 32 | 32 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 13 | 30 | 31 | 31 | 31 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 20 | 24 | 25 | 26 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 4.1 | 13.2 | 47.6 | 61.1 | 64.5 | 68.3 |

*C. freundii* FRHCFR2 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 5 | 10 | 17 | 23 | 25 | 26 | 28 | 29 |
| 10 | 0 | 0 | 0 | 0 | 0 | 4 | 6 | 9 | 10 | 11 | 11 | 12 | 13 |
| 5 | 0 | 0 | 0 | 0 | 0 | 2 | 5 | 8 | 10 | 11 | 11 | 11 | 11 |
| MPN | 0 | 0 | 0 | 0 | 0 | 2.1 | 4.3 | 7.9 | 11.2 | 12.9 | 13.5 | 15.1 | 16.3 |

*K. pneumoniae* FRHKPN2 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 8 | 24 | 30 | 31 | 31 | 31 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 11 | 13 | 20 | 29 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 6 | 11 | 16 | 18 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 1.5 | 8.1 | 13.9 | 17.8 | 25.5 | 38.3 |

*E. cloacae* FRHECL2 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 14 | 31 | 32 | 32 | 32 | 32 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 14 | 15 | 22 | 32 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 5 | 12 | 24 | 31 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0.7 | 2.9 | 8.7 | 16.4 | 20.4 | 36.5 | 140.9 |

*E. aerogenes* FRHEAE2 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 9 | 26 | 31 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 18 | 32 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 28 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 0.5 | 2.2 | 15.9 | 70 |

## Appendix 5.5 (Cont'd..): Pattern of positive/negative wells of EHC-GAL, 4-MU-GAL and LTB with 10 coliform organisms.

*C. freundii* NCTC 9750 with 4-MU-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h | LTB 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 6 | 29 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 23 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 10 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 2.3 | 21.8 |

*K. pneumoniae* NCTC 10896 with 4-MU-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 1 | 3 | 13 | 14 | 17 | 17 | 25 | 26 | 26 | 31 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 8 | 14 | 14 | 20 | 22 | 23 | 23 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 3 | 12 | 12 | 17 | 18 |
| MPN | 0 | 0 | 0 | 0 | 0.2 | 0.6 | 4.2 | 5.2 | 5.3 | 5.3 | 17.4 | 19.3 | 22.5 | 29.9 |

*E. cloacae* NCTC 11936 with 4-MU-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 2 | 14 | 17 | 18 | 18 | 18 | 19 | 19 | 26 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 8 | 12 | 12 | 13 | 13 | 13 | 23 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 5 | 5 | 5 | 5 | 5 | 20 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0.4 | 4.5 | 6.6 | 8.4 | 8.4 | 8.7 | 9 | 9 | 24.1 |

*E. coli* NCIMB 10213 with 4-MU-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12.3 |

*E. aerogenes* NCIMB 10102 with 4-MU-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 16 | 28 | 31 | 32 | 32 | 32 | 32 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 18 | 26 | 28 | 28 | 29 | 29 | 32 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 11 | 20 | 22 | 24 | 24 | 24 | 30 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 4.4 | 11.2 | 29.5 | 41.6 | 49.3 | 52.6 | 52.6 | 115 |

*C. freundii* FRHCFR2 with 4-MU-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 10 | 16 | 17 | 18 | 18 | 19 | 28 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 6 | 9 | 10 | 11 | 11 | 11 | 27 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 14 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0.2 | 0.7 | 3.3 | 6.3 | 6.9 | 7.5 | 7.5 | 7.9 | 26.2 |

*K. pneumoniae* FRHKPN2 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 20 | 25 | 28 | 31 | 32 | 31 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 24 | 26 | 26 | 27 | 27 | 31 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 23 | 24 | 24 | 25 | 25 | 19 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.3 | 20.5 | 27.7 | 32.7 | 43.7 | 48.3 | 43.7 |

*E. cloacae* FRHECL2 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 24 | 28 | 32 | 32 | 32 | 32 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 19 | 29 | 31 | 31 | 31 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 18 | 26 | 26 | 29 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 9.9 | 15.6 | 41.7 | 68.3 | 68.3 | 84.1 |

*E. aerogenes* FRHEAE2 with EHC-GAL

| | 0 | 360 | 390 | 420 | 450 | 480 | 510 | 540 | 570 | 600 | 630 | 660 | 24h | 24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 7 | 7 | 17 | 32 | 32 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 7 | 7 | 17 | 32 | 30 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 4 | 3 | 28 | 27 |
| MPN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 9 | 90.3 | 65.7 |

## Appendix 5.6: Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with raw sewage sample over 18 h.

EHC-GAL

| Dilution | Replicate | 0 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 0 | 4135 | 4349 | 4617 | 5096 | 6024 | 6403 | 7611 | 8264 | 9381 | 11490 | 15714 | 22248 | 29996 | 37922 |
| neat | 2 | 0 | 3901 | 4129 | 4364 | 4935 | 5973 | 6299 | 7413 | 8121 | 8967 | 11274 | 15189 | 22019 | 30079 | 37095 |
| neat | 3 | 0 | 4502 | 4603 | 4777 | 5216 | 6193 | 6504 | 7590 | 8332 | 9290 | 11393 | 15061 | 20399 | 28428 | 36906 |
| neat | 4 | 0 | 4101 | 4367 | 4498 | 5221 | 6121 | 6271 | 7547 | 8069 | 9137 | 11587 | 16147 | 23111 | 31723 | 39951 |
| neat | 5 | 0 | 3797 | 4004 | 4227 | 4831 | 5704 | 5979 | 7038 | 7834 | 8609 | 10715 | 13941 | 18928 | 24973 | 31345 |
| neat | 6 | 0 | 3598 | 3687 | 3919 | 4428 | 5362 | 5731 | 6684 | 7346 | 7886 | 9885 | 13459 | 19101 | 26749 | 34238 |
| neat | 7 | 0 | 3677 | 4059 | 4208 | 4907 | 5621 | 6128 | 7141 | 7828 | 8450 | 10184 | 12860 | 17026 | 22733 | 29004 |
| -1 | 1 | 0 | 449 | 424 | 363 | 443 | 696 | 678 | 1016 | 1090 | 1187 | 1495 | 2264 | 4343 | 8560 | 15335 |
| -1 | 2 | 0 | 201 | 198 | 107 | 171 | 458 | 424 | 699 | 763 | 744 | 1016 | 1477 | 2807 | 5084 | 8929 |
| -1 | 3 | 0 | 317 | 311 | 247 | 302 | 528 | 482 | 882 | 824 | 833 | 1123 | 1379 | 2276 | 4828 | 10876 |
| -1 | 4 | 0 | 403 | 412 | 210 | 442 | 692 | 546 | 918 | 876 | 900 | 1071 | 1596 | 2258 | 3781 | 6454 |
| -1 | 5 | 0 | 452 | 385 | 324 | 464 | 678 | 650 | 934 | 1062 | 1077 | 1425 | 1944 | 3391 | 6317 | 11173 |
| -1 | 6 | 0 | 363 | 247 | 201 | 296 | 589 | 629 | 839 | 973 | 854 | 1111 | 1541 | 2374 | 4172 | 6714 |
| -1 | 7 | 0 | 119 | 168 | 61 | 296 | 372 | 507 | 653 | 732 | 711 | 928 | 1166 | 1825 | 3555 | 7074 |
| -2 | 1 | 0 | -119 | -192 | -412 | -278 | -174 | -409 | -244 | -122 | -333 | -198 | -201 | -323 | -329 | -302 |
| -2 | 2 | 0 | -366 | -393 | -522 | -522 | -351 | -448 | -311 | -338 | -451 | -393 | -412 | -250 | -164 | -290 |
| -2 | 3 | 0 | -244 | -277 | -366 | -363 | -265 | -372 | -39 | -244 | -308 | -174 | -262 | -125 | -82 | -134 |
| -2 | 4 | 0 | -174 | -195 | -437 | -311 | -180 | -394 | -119 | -302 | -363 | -354 | -199 | -266 | -214 | 64 |
| -2 | 5 | 0 | -171 | -250 | -372 | -330 | -250 | -345 | -214 | -214 | -314 | -214 | -253 | -150 | 214 | 717 |
| -2 | 6 | 0 | -132 | -306 | -406 | -403 | -217 | -257 | -187 | -104 | -348 | -254 | -190 | -132 | 183 | 839 |
| -2 | 7 | 0 | -219 | -311 | -436 | -305 | -293 | -265 | -238 | -259 | -329 | -274 | -256 | -244 | 0 | 953 |
| -3 | 1 | 0 | -207 | -235 | -491 | -357 | -265 | -519 | -375 | -274 | -497 | -363 | -384 | -378 | -375 | -543 |
| -3 | 2 | 0 | -376 | -394 | -553 | -577 | -434 | -519 | -412 | -446 | -571 | -541 | -580 | -568 | -467 | -559 |
| -3 | 3 | 0 | -357 | -387 | -497 | -497 | -418 | -525 | -256 | -326 | -503 | -418 | -512 | -451 | -360 | -522 |
| -3 | 4 | 0 | -257 | -300 | -477 | -358 | -242 | -470 | -211 | -409 | -480 | -461 | -214 | 607 | 2349 | 5285 |
| -3 | 5 | 0 | -213 | -305 | -436 | -396 | -332 | -430 | -311 | -335 | -439 | -351 | -448 | -439 | -375 | -354 |
| -3 | 6 | 0 | -229 | -341 | -470 | -473 | -277 | -326 | -299 | -296 | -497 | -433 | -415 | -503 | -503 | -525 |
| -3 | 7 | 0 | -165 | -308 | -424 | -311 | -271 | -305 | -299 | -329 | -424 | -384 | -281 | -302 | -256 | 6 |
| -4 | 1 | 0 | -269 | -339 | -540 | -446 | -369 | -571 | -449 | -385 | -562 | -452 | -528 | -473 | -558 | -616 |
| -4 | 2 | 0 | -339 | -321 | -562 | -571 | -433 | -504 | -418 | -452 | -592 | -559 | -583 | -589 | -580 | -556 |
| -4 | 3 | 0 | -400 | -315 | -510 | -434 | -440 | -544 | -330 | -293 | -535 | -480 | -553 | -547 | -507 | -586 |
| -4 | 4 | 0 | -338 | -369 | -476 | -381 | -253 | -503 | -219 | -421 | -506 | -524 | -534 | -463 | -500 | -415 |
| -4 | 5 | 0 | -275 | -358 | -477 | -446 | -394 | -489 | -376 | -412 | -519 | -422 | -538 | -528 | -525 | -483 |
| -4 | 6 | 0 | -345 | -351 | -507 | -501 | -297 | -528 | -297 | -449 | -541 | -455 | -416 | -538 | -553 | -580 |
| -4 | 7 | 0 | -137 | -329 | -448 | -338 | -213 | -351 | -323 | -375 | -457 | -421 | -360 | -348 | -326 | -427 |
| -5 | 1 | 0 | -357 | -436 | -623 | -516 | -458 | -620 | -501 | -470 | -632 | -552 | -601 | -580 | -653 | -656 |
| -5 | 2 | 0 | -323 | -302 | -574 | -574 | -424 | -488 | -418 | -433 | -598 | -564 | -592 | -607 | -586 | -497 |
| -5 | 3 | 0 | -451 | -344 | -582 | -451 | -503 | -607 | -424 | -427 | -616 | -564 | -628 | -622 | -592 | -616 |
| -5 | 4 | 0 | -326 | -372 | -470 | -366 | -280 | -506 | -229 | -412 | -470 | -412 | -61 | 1267 | 3986 | 9467 |
| -5 | 5 | 0 | -299 | -406 | -522 | -473 | -437 | -534 | -430 | -455 | -565 | -415 | -571 | -577 | -574 | -571 |
| -5 | 6 | 0 | -342 | -266 | -501 | -486 | -278 | -516 | -239 | -428 | -519 | -431 | -550 | -474 | -458 | -538 |
| -5 | 7 | 0 | -165 | -351 | -461 | -317 | -244 | -375 | -351 | -397 | -479 | -418 | -415 | -415 | -409 | -485 |
| neg | 1 | 0 | -336 | -351 | -474 | -379 | -251 | -504 | -290 | -419 | -495 | -516 | -528 | -400 | -379 | -513 |
| neg | 2 | 0 | -336 | -363 | -491 | -382 | -244 | -519 | -385 | -403 | -510 | -507 | -552 | -540 | -430 | -556 |
| neg | 3 | 0 | -320 | -332 | -470 | -354 | -228 | -494 | -372 | -366 | -503 | -503 | -524 | -546 | -537 | -540 |
| neg | 4 | 0 | -302 | -306 | -461 | -312 | -220 | -483 | -370 | -318 | -492 | -483 | -525 | -528 | -528 | -531 |
| neg | 5 | 0 | -286 | -219 | -466 | -326 | -357 | -488 | -393 | -268 | -488 | -506 | -531 | -527 | -531 | -527 |
| neg | 6 | 0 | -216 | -189 | -433 | -436 | -363 | -448 | -360 | -290 | -470 | -460 | -476 | -494 | -473 | -460 |

**Appendix 5.6 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with raw sewage sample over 18 h.**

EHC-GAL

| Dilution | Replicate | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 | 14 | 14.5 | 15 | 15.5 | 16 | 16.5 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 44633 | 49925 | 53291 | 54951 | 56132 | 56737 | 56724 | 56593 | 56090 | 56688 | 56450 | 56337 | 56550 | 56962 | 57060 |
| neat | 2 | 43599 | 47575 | 50624 | 51970 | 53179 | 53584 | 53786 | 53975 | 53966 | 54314 | 54610 | 54073 | 54680 | 54469 | 54830 |
| neat | 3 | 43751 | 48995 | 52565 | 53444 | 54399 | 54372 | 55016 | 55586 | 55352 | 55248 | 55287 | 54799 | 55309 | 55352 | 55385 |
| neat | 4 | 45664 | 49314 | 51698 | 52344 | 53468 | 53178 | 53980 | 53791 | 54133 | 53532 | 53681 | 54316 | 54298 | 53889 | 54343 |
| neat | 5 | 38117 | 43272 | 47529 | 50071 | 52400 | 54057 | 54292 | 55064 | 55293 | 55180 | 55708 | 55675 | 56227 | 56532 | 55806 |
| neat | 6 | 40541 | 45893 | 49498 | 51567 | 52806 | 53084 | 54277 | 53859 | 54399 | 54408 | 54793 | 54692 | 54347 | 54439 | 54521 |
| neat | 7 | 34772 | 40522 | 44972 | 47877 | 50422 | 51435 | 52867 | 53798 | 54368 | 54445 | 54469 | 54557 | 55235 | 55372 | 55171 |
| -1 | 1 | 23945 | 33091 | 41126 | 46782 | 51207 | 54139 | 55610 | 56666 | 56636 | 57661 | 58153 | 57680 | 58073 | 58497 | 58687 |
| -1 | 2 | 15271 | 22684 | 30625 | 37806 | 43275 | 47563 | 50321 | 52519 | 53721 | 54777 | 55702 | 55439 | 56297 | 56321 | 56575 |
| -1 | 3 | 19712 | 30994 | 41538 | 48655 | 53196 | 55162 | 55738 | 56636 | 56147 | 56324 | 56236 | 56160 | 56504 | 56660 | 56880 |
| -1 | 4 | 10028 | 14691 | 19733 | 24616 | 29819 | 34229 | 39103 | 42503 | 45655 | 47599 | 49522 | 50950 | 52589 | 52946 | 54136 |
| -1 | 5 | 17435 | 23875 | 29917 | 35987 | 41005 | 45457 | 48591 | 51500 | 53157 | 54256 | 55308 | 55983 | 57121 | 57567 | 57484 |
| -1 | 6 | 10422 | 15305 | 20731 | 26770 | 32914 | 38792 | 44447 | 47990 | 50941 | 52909 | 54051 | 54576 | 54875 | 55125 | 55406 |
| -1 | 7 | 12152 | 19370 | 26554 | 34119 | 41227 | 46586 | 49897 | 52632 | 53645 | 54313 | 55107 | 55259 | 56071 | 56315 | 56578 |
| -2 | 1 | -281 | -241 | -180 | 61 | 37 | 281 | 745 | 1074 | 1834 | 2710 | 3928 | 5628 | 7556 | 13785 | 25629 |
| -2 | 2 | -113 | 3 | 455 | 1010 | 1776 | 2961 | 4425 | 6730 | 10236 | 15168 | 21262 | 27287 | 33177 | 38017 | 41841 |
| -2 | 3 | 98 | 351 | 1142 | 2579 | 4990 | 8744 | 13865 | 19254 | 24726 | 30919 | 36836 | 42683 | 46635 | 49873 | 52541 |
| -2 | 4 | 561 | 1770 | 4285 | 7962 | 12961 | 18497 | 25101 | 31305 | 37409 | 42106 | 46617 | 49324 | 51820 | 52955 | 54191 |
| -2 | 5 | 1688 | 3690 | 6632 | 10947 | 15741 | 21457 | 27268 | 32343 | 37028 | 41468 | 45371 | 49040 | 52314 | 54075 | 55183 |
| -2 | 6 | 2276 | 4828 | 8102 | 12579 | 17636 | 23190 | 28653 | 33545 | 38089 | 42966 | 46424 | 49210 | 51676 | 53031 | 53901 |
| -2 | 7 | 2720 | 6220 | 11384 | 18678 | 27742 | 38542 | 45860 | 50646 | 52104 | 53502 | 54363 | 54726 | 55028 | 55202 | 55467 |
| -3 | 1 | -522 | -531 | -516 | -458 | -451 | -506 | -427 | -537 | -384 | -534 | -534 | -421 | -454 | -488 | -509 |
| -3 | 2 | -452 | -583 | -553 | -412 | -327 | 164 | 1055 | 3006 | 6064 | 10443 | 15811 | 21933 | 28128 | 34052 | 39414 |
| -3 | 3 | -375 | -512 | -485 | -378 | -497 | -485 | -360 | -464 | -488 | -473 | -323 | -421 | -467 | -467 | -476 |
| -3 | 4 | 9570 | 15747 | 24359 | 36774 | 47858 | 52128 | 53211 | 53739 | 53553 | 53291 | 53870 | 53400 | 53800 | 53709 | 53687 |
| -3 | 5 | -384 | -369 | -351 | -311 | -232 | -55 | 351 | 672 | 1362 | 2601 | 4294 | 7209 | 11661 | 16816 | 21867 |
| -3 | 6 | -491 | -381 | -500 | -482 | -491 | -433 | -476 | -479 | -488 | -424 | -454 | -470 | -427 | -436 | -311 |
| -3 | 7 | 400 | 1190 | 2481 | 4227 | 6717 | 10651 | 16221 | 22623 | 28867 | 35847 | 42317 | 47886 | 51457 | 53603 | 55223 |
| -4 | 1 | -598 | -607 | -565 | -595 | -467 | -461 | -574 | -632 | -534 | -650 | -650 | -626 | -516 | -482 | -684 |
| -4 | 2 | -461 | -611 | -617 | -620 | -604 | -629 | -632 | -525 | -592 | -641 | -656 | -632 | -641 | -565 | -672 |
| -4 | 3 | -516 | -571 | -446 | -544 | -440 | -599 | -565 | -583 | -470 | -638 | -580 | -608 | -467 | -452 | -611 |
| -4 | 4 | -534 | -524 | -512 | -430 | -500 | -445 | -268 | 275 | 1395 | 3275 | 6464 | 10694 | 16013 | 22184 | 28077 |
| -4 | 5 | -531 | -544 | -391 | -535 | -547 | -528 | -504 | -547 | -525 | -351 | -272 | -80 | -49 | 262 | 686 |
| -4 | 6 | -550 | -583 | -562 | -577 | -586 | -440 | -580 | -577 | -577 | -519 | -522 | -556 | -431 | -394 | -391 |
| -4 | 7 | -296 | -390 | -244 | -15 | 827 | 2210 | 5619 | 11112 | 18339 | 27839 | 37260 | 45784 | 51082 | 53767 | 55312 |
| -5 | 1 | -498 | -647 | -540 | -635 | -577 | -528 | -644 | -525 | -626 | -696 | -571 | -684 | -574 | -565 | -717 |
| -5 | 2 | -537 | -598 | -580 | -589 | -561 | -622 | -574 | -476 | -537 | -626 | -629 | -622 | -662 | -531 | -668 |
| -5 | 3 | -576 | -582 | -521 | -586 | -521 | -598 | -582 | -582 | -494 | -604 | -610 | -619 | -479 | -521 | -619 |
| -5 | 4 | 20094 | 37919 | 49876 | 52910 | 53407 | 53740 | 53639 | 54155 | 53316 | 53502 | 53474 | 53749 | 53508 | 54164 | 53529 |
| -5 | 5 | -577 | -592 | -543 | -598 | -443 | -583 | -598 | -623 | -638 | -525 | -586 | -522 | -647 | -647 | -668 |
| -5 | 6 | -510 | -553 | -461 | -544 | -528 | -568 | -577 | -547 | -596 | -501 | -458 | -617 | -464 | -483 | -638 |
| -5 | 7 | -424 | -516 | -507 | -516 | -421 | -516 | -482 | -504 | -519 | -366 | -528 | -507 | -491 | -436 | -375 |
| neg | 1 | -516 | -522 | -495 | -565 | -538 | -498 | -550 | -431 | -562 | -562 | -571 | -568 | -593 | -495 | -605 |
| neg | 2 | -540 | -540 | -464 | -556 | -549 | -455 | -546 | -406 | -592 | -592 | -562 | -556 | -604 | -461 | -620 |
| neg | 3 | -485 | -509 | -473 | -543 | -506 | -399 | -473 | -543 | -570 | -564 | -424 | -488 | -582 | -598 | -592 |
| neg | 4 | -452 | -479 | -537 | -479 | -464 | -519 | -400 | -556 | -556 | -565 | -577 | -415 | -565 | -592 | -559 |
| neg | 5 | -393 | -396 | -540 | -427 | -393 | -579 | -555 | -576 | -555 | -579 | -592 | -589 | -521 | -592 | -497 |
| neg | 6 | -518 | -479 | -482 | -522 | -522 | -543 | -540 | -522 | -460 | -558 | -522 | -586 | -424 | -543 | -473 |

**Appendix 5.6 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with raw sewage sample over 18 h.**

EHC-GAL

| Dilution | Replicate | 17.5 | 18 |
|---|---|---|---|
| neat | 1 | 56584 | 57322 |
| neat | 2 | 54711 | 54363 |
| neat | 3 | 55055 | 55071 |
| neat | 4 | 54386 | 54527 |
| neat | 5 | 56038 | 56145 |
| neat | 6 | 54152 | 54841 |
| neat | 7 | 55168 | 55195 |
| -1 | 1 | 58064 | 58662 |
| -1 | 2 | 56428 | 56605 |
| -1 | 3 | 56453 | 56468 |
| -1 | 4 | 54539 | 55094 |
| -1 | 5 | 57542 | 57600 |
| -1 | 6 | 55110 | 55812 |
| -1 | 7 | 56477 | 56325 |
| -2 | 1 | 34797 | 41297 |
| -2 | 2 | 44578 | 47249 |
| -2 | 3 | 53627 | 54613 |
| -2 | 4 | 54454 | 55250 |
| -2 | 5 | 55928 | 56477 |
| -2 | 6 | 54044 | 54710 |
| -2 | 7 | 55351 | 55452 |
| -3 | 1 | -509 | -430 |
| -3 | 2 | 44416 | 48707 |
| -3 | 3 | -503 | -454 |
| -3 | 4 | 53159 | 53864 |
| -3 | 5 | 26380 | 30204 |
| -3 | 6 | -467 | -332 |
| -3 | 7 | 55617 | 55946 |
| -4 | 1 | -589 | -626 |
| -4 | 2 | -662 | -531 |
| -4 | 3 | -498 | -516 |
| -4 | 4 | 34083 | 39857 |
| -4 | 5 | 1294 | 2145 |
| -4 | 6 | -254 | 27 |
| -4 | 7 | 55849 | 56206 |
| -5 | 1 | -577 | -678 |
| -5 | 2 | -687 | -546 |
| -5 | 3 | -488 | -540 |
| -5 | 4 | 53551 | 54079 |
| -5 | 5 | -653 | -656 |
| -5 | 6 | -583 | -654 |
| -5 | 7 | -284 | 58 |
| neg | 1 | -614 | -568 |
| neg | 2 | -644 | -589 |
| neg | 3 | -604 | -540 |
| neg | 4 | -568 | -495 |
| neg | 5 | -497 | -485 |
| neg | 6 | -503 | -464 |

**Appendix 5.6 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with raw sewage sample over 18 h.**

**4-MU-GAL**

| Dilution | Replicate | 0 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 0 | 17255 | 17218 | 17157 | 17108 | 17059 | 17032 | 16998 | 16971 | 16949 | 17273 | 18744 | 21332 | 24310 | 27176 |
| neat | 2 | 0 | 2542 | 2862 | 2969 | 3421 | 3961 | 4385 | 4922 | 5523 | 5945 | 7531 | 10437 | 15448 | 21262 | 27683 |
| neat | 3 | 0 | 2457 | 2768 | 3177 | 3595 | 4077 | 4346 | 5008 | 5506 | 5741 | 6903 | 8838 | 13205 | 19108 | 26225 |
| neat | 4 | 0 | 2374 | 2731 | 2911 | 3314 | 3934 | 4184 | 4749 | 5331 | 5725 | 7013 | 10172 | 15247 | 21897 | 28205 |
| neat | 5 | 0 | 2234 | 2603 | 2945 | 3213 | 3754 | 4181 | 4657 | 5093 | 5826 | 7462 | 10498 | 15137 | 21009 | 27509 |
| neat | 6 | 0 | 2369 | 2720 | 3128 | 3421 | 3928 | 4154 | 4834 | 5320 | 5811 | 6949 | 9476 | 14176 | 21201 | 28398 |
| neat | 7 | 0 | 2197 | 2499 | 2777 | 3152 | 3695 | 4001 | 4547 | 5075 | 5462 | 6619 | 9262 | 13327 | 18875 | 24924 |
| -1 | 1 | 0 | 205 | 180 | 140 | 214 | 330 | 327 | 482 | 546 | 739 | 797 | 1151 | 1981 | 3842 | 6955 |
| -1 | 2 | 0 | 183 | 231 | 134 | 189 | 311 | 442 | 463 | 698 | 631 | 906 | 1290 | 2011 | 3180 | 5212 |
| -1 | 3 | 0 | 186 | 229 | 382 | 430 | 458 | 479 | 659 | 897 | 836 | 1264 | 1981 | 3516 | 6345 | 10623 |
| -1 | 4 | 0 | 86 | 205 | 101 | 150 | 327 | 342 | 382 | 562 | 449 | 507 | 775 | 1077 | 2008 | 3607 |
| -1 | 5 | 0 | 131 | 147 | 290 | 198 | 311 | 464 | 455 | 525 | 607 | 815 | 1532 | 3046 | 6015 | 10932 |
| -1 | 6 | 0 | 37 | 65 | 181 | 147 | 196 | 177 | 306 | 382 | 483 | 522 | 550 | 797 | 1465 | 2942 |
| -1 | 7 | 0 | 104 | 147 | 143 | 195 | 311 | 363 | 446 | 635 | 568 | 696 | 1273 | 2246 | 4529 | 9354 |
| -2 | 1 | 0 | -189 | -217 | -101 | -91 | -204 | -88 | -88 | -101 | -192 | -210 | -64 | -64 | -140 | -116 |
| -2 | 2 | 0 | -77 | 24 | -156 | -132 | -49 | 58 | 6 | 146 | 6 | 48 | 73 | 125 | 253 | 735 |
| -2 | 3 | 0 | -244 | -101 | -180 | -211 | -238 | -269 | -201 | -61 | -223 | -110 | -27 | -107 | -21 | 278 |
| -2 | 4 | 0 | -192 | -98 | -244 | -238 | -159 | -104 | -153 | -21 | -171 | -180 | -43 | -55 | -104 | -9 |
| -2 | 5 | 0 | -183 | -207 | -82 | -232 | -180 | -110 | -159 | -143 | -143 | -171 | -21 | -88 | -110 | 15 |
| -2 | 6 | 0 | -219 | -225 | -106 | -210 | -204 | -222 | -180 | -158 | -137 | -103 | -164 | -170 | -155 | -140 |
| -2 | 7 | 0 | -211 | -208 | -220 | -238 | -186 | -171 | -153 | -95 | -192 | -162 | 0 | 3 | -141 | 3 |
| -3 | 1 | 0 | -250 | -143 | -256 | -235 | -159 | -281 | -232 | -244 | -293 | -296 | -256 | -229 | -232 | -195 |
| -3 | 2 | 0 | -378 | -262 | -418 | -449 | -403 | -345 | -403 | -269 | -436 | -424 | -403 | -415 | -433 | -403 |
| -3 | 3 | 0 | -336 | -210 | -275 | -329 | -348 | -406 | -339 | -268 | -320 | -275 | -241 | -357 | -369 | -339 |
| -3 | 4 | 0 | -259 | -113 | -317 | -320 | -152 | -207 | -265 | -131 | -287 | -323 | -177 | -213 | -302 | -302 |
| -3 | 5 | 0 | -242 | -266 | -162 | -306 | -269 | -269 | -266 | -272 | -263 | -300 | -159 | -199 | -272 | -293 |
| -3 | 6 | 0 | -382 | -406 | -269 | -394 | -397 | -418 | -382 | -376 | -357 | -339 | -412 | -412 | -409 | -269 |
| -3 | 7 | 0 | -214 | -226 | -260 | -266 | -232 | -208 | -195 | -214 | -247 | -257 | -104 | -101 | -250 | -116 |
| -4 | 1 | 0 | -268 | -220 | -308 | -293 | -174 | -317 | -268 | -284 | -320 | -293 | -311 | -302 | -171 | -195 |
| -4 | 2 | 0 | -326 | -216 | -274 | -421 | -378 | -290 | -363 | -222 | -390 | -387 | -354 | -360 | -360 | -396 |
| -4 | 3 | 0 | -250 | -180 | -186 | -265 | -274 | -311 | -259 | -189 | -149 | -241 | -219 | -302 | -238 | -299 |
| -4 | 4 | 0 | -385 | -327 | -434 | -449 | -370 | -351 | -391 | -257 | -428 | -431 | -302 | -333 | -431 | -437 |
| -4 | 5 | 0 | -250 | -284 | -199 | -324 | -290 | -327 | -284 | -302 | -232 | -321 | -321 | -269 | -165 | -293 |
| -4 | 6 | 0 | -242 | -281 | -156 | -281 | -275 | -306 | -272 | -266 | -269 | -208 | -278 | -242 | 6 | 231 |
| -4 | 7 | 0 | -259 | -256 | -305 | -308 | -281 | -311 | -262 | -266 | -305 | -311 | -275 | -281 | -278 | -308 |
| -5 | 1 | 0 | -265 | -284 | -329 | -317 | -256 | -342 | -271 | -293 | -317 | -180 | -339 | -326 | -305 | -293 |
| -5 | 2 | 0 | -302 | -186 | -244 | -369 | -323 | -216 | -320 | -271 | -363 | -357 | -311 | -256 | -241 | -329 |
| -5 | 3 | 0 | -250 | -235 | -192 | -265 | -281 | -317 | -265 | -210 | -162 | -207 | -241 | -302 | -183 | -290 |
| -5 | 4 | 0 | -320 | -320 | -378 | -381 | -342 | -290 | -345 | -189 | -375 | -369 | -217 | -244 | -326 | -339 |
| -5 | 5 | 0 | -223 | -266 | -250 | -305 | -278 | -308 | -263 | -281 | -165 | -302 | -312 | -308 | -312 | -226 |
| -5 | 6 | 0 | -222 | -280 | -228 | -247 | -280 | -314 | -271 | -253 | -268 | -207 | -299 | -308 | -262 | -235 |
| -5 | 7 | 0 | -330 | -309 | -373 | -388 | -354 | -391 | -348 | -354 | -376 | -394 | -382 | -370 | -238 | -388 |
| neg | 1 | 0 | -311 | -320 | -369 | -360 | -314 | -360 | -165 | -278 | -195 | -241 | -357 | -360 | -351 | -336 |
| neg | 2 | 0 | -156 | -275 | -320 | -314 | -287 | -171 | -131 | -131 | -171 | -265 | -314 | -320 | -253 | -302 |
| neg | 3 | 0 | -104 | -125 | -320 | -323 | -290 | -247 | -204 | -210 | -232 | -290 | -177 | -311 | -247 | -277 |
| neg | 4 | 0 | -116 | -155 | -335 | -335 | -302 | -299 | -259 | -244 | -283 | -289 | -192 | -338 | -317 | -174 |
| neg | 5 | 0 | -165 | -278 | -290 | -378 | -339 | -363 | -305 | -299 | -345 | -357 | -299 | -327 | -381 | -278 |
| neg | 6 | 0 | -229 | -357 | -302 | -434 | -394 | -434 | -379 | -369 | -409 | -379 | -366 | -415 | -437 | -369 |

**Appendix 5.6 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with raw sewage sample over 18 h.**

**4-MU-GAL**

| Dilution | Replicate | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 | 14 | 14.5 | 15 | 15.5 | 16 | 16.5 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 30158 | 32303 | 33722 | 34085 | 34098 | 34079 | 34052 | 34027 | 33976 | 33985 | 33963 | 33966 | 34098 | 34259 | 34281 |
| neat | 2 | 32975 | 36860 | 40195 | 41852 | 42252 | 43433 | 43876 | 44141 | 44449 | 44996 | 44895 | 45243 | 45554 | 45911 | 46146 |
| neat | 3 | 33027 | 36839 | 40123 | 41215 | 41703 | 42399 | 42677 | 43336 | 43388 | 43675 | 44157 | 44712 | 44987 | 45259 | 45674 |
| neat | 4 | 33597 | 37006 | 38523 | 39478 | 39539 | 40122 | 40019 | 40247 | 40843 | 41377 | 42237 | 42646 | 43363 | 44196 | 44279 |
| neat | 5 | 32993 | 36308 | 39304 | 40366 | 41468 | 41645 | 42292 | 42875 | 43006 | 43656 | 44010 | 44532 | 45005 | 45176 | 45320 |
| neat | 6 | 34010 | 37492 | 39781 | 40510 | 40730 | 41334 | 41655 | 41978 | 42097 | 42424 | 43089 | 43098 | 43901 | 44066 | 44252 |
| neat | 7 | 30789 | 35019 | 38044 | 40009 | 40360 | 40683 | 41355 | 41114 | 41715 | 41755 | 42475 | 42771 | 43082 | 43137 | 43168 |
| -1 | 1 | 11899 | 18772 | 26911 | 33598 | 37953 | 40599 | 42005 | 42692 | 43229 | 43388 | 44053 | 44773 | 44432 | 44838 | 44828 |
| -1 | 2 | 7971 | 12118 | 17560 | 23120 | 28327 | 33371 | 36844 | 38859 | 40839 | 41886 | 42597 | 43116 | 43729 | 44150 | 44129 |
| -1 | 3 | 15979 | 22211 | 28425 | 33311 | 36961 | 39817 | 40632 | 41529 | 41774 | 42466 | 43000 | 43299 | 43342 | 43580 | 43959 |
| -1 | 4 | 6595 | 11982 | 19898 | 29523 | 37434 | 41508 | 42228 | 42536 | 43119 | 43296 | 44078 | 44151 | 44621 | 45118 | 45100 |
| -1 | 5 | 17106 | 23047 | 29029 | 33323 | 36305 | 37928 | 38600 | 39329 | 39610 | 40092 | 40498 | 40849 | 41133 | 41267 | 41545 |
| -1 | 6 | 6028 | 10700 | 18037 | 26222 | 32536 | 36766 | 39067 | 40172 | 40520 | 40941 | 41582 | 41591 | 42378 | 42515 | 42561 |
| -1 | 7 | 19401 | 30454 | 35160 | 37800 | 38835 | 39891 | 41084 | 41032 | 41697 | 41987 | 42262 | 42759 | 43022 | 43302 | 43141 |
| -2 | 1 | -24 | 198 | 348 | 684 | 1178 | 1892 | 2985 | 4526 | 6949 | 10389 | 14377 | 19037 | 23554 | 27460 | 31550 |
| -2 | 2 | 1852 | 3933 | 7074 | 10785 | 15121 | 20197 | 26273 | 31494 | 36145 | 38562 | 40372 | 40946 | 41669 | 41965 | 42057 |
| -2 | 3 | 882 | 2417 | 5139 | 9217 | 14209 | 19385 | 24253 | 28992 | 33161 | 36213 | 38200 | 39173 | 39546 | 39933 | 40397 |
| -2 | 4 | 189 | 729 | 1520 | 3159 | 6656 | 12131 | 18570 | 24888 | 30061 | 33601 | 36387 | 38389 | 39784 | 40919 | 41502 |
| -2 | 5 | 315 | 705 | 1618 | 2829 | 4544 | 7026 | 10154 | 14014 | 18906 | 23847 | 28025 | 32011 | 34724 | 37214 | 38887 |
| -2 | 6 | 19 | 10 | 65 | 577 | 1767 | 4395 | 9205 | 16273 | 24174 | 30400 | 35100 | 38121 | 40205 | 41124 | 41551 |
| -2 | 7 | -98 | -28 | 104 | 345 | 1187 | 3326 | 7724 | 13269 | 19657 | 25647 | 30176 | 33396 | 35871 | 37833 | 38624 |
| -3 | 1 | 70 | 86 | 418 | 891 | 1889 | 3183 | 5319 | 8176 | 12827 | 18647 | 24763 | 29917 | 33988 | 37107 | 38655 |
| -3 | 2 | -424 | -293 | -384 | -284 | -406 | -333 | -330 | -394 | -339 | -372 | -253 | -363 | -354 | -336 | -342 |
| -3 | 3 | -342 | -262 | -192 | 110 | 366 | 864 | 1764 | 2777 | 4450 | 6552 | 9195 | 12513 | 16453 | 21158 | 25611 |
| -3 | 4 | -280 | -146 | -137 | -280 | -241 | -195 | -265 | -250 | -232 | -210 | -158 | -76 | 64 | 272 | 385 |
| -3 | 5 | -211 | -275 | -141 | -150 | -281 | -266 | -278 | -275 | -254 | -159 | -263 | -190 | -248 | -248 | -242 |
| -3 | 6 | -330 | -257 | -397 | -238 | -278 | -241 | -370 | -348 | -196 | -202 | -235 | -171 | -296 | -309 | -156 |
| -3 | 7 | -226 | -144 | 18 | 143 | 778 | 1926 | 3897 | 6149 | 9024 | 12369 | 15851 | 19065 | 22245 | 25415 | 27610 |
| -4 | 1 | -293 | -305 | -308 | -226 | -287 | -162 | -284 | -198 | -284 | -284 | -265 | -223 | -204 | -140 | -101 |
| -4 | 2 | -396 | -271 | -372 | -378 | -375 | -381 | -247 | -375 | -229 | -384 | -357 | -375 | -363 | -308 | -351 |
| -4 | 3 | -302 | -305 | -131 | -256 | -296 | -280 | -238 | -149 | -277 | -274 | -274 | -277 | -155 | -149 | -134 |
| -4 | 4 | -382 | -421 | -418 | -409 | -345 | -251 | -406 | -363 | -367 | -412 | -418 | -370 | -345 | -397 | -296 |
| -4 | 5 | -235 | -247 | -302 | -315 | -311 | -253 | -302 | -287 | -180 | -174 | -278 | -141 | -281 | -269 | -266 |
| -4 | 6 | 726 | 2002 | 4367 | 8160 | 13730 | 20541 | 26782 | 32355 | 36338 | 38541 | 39765 | 40491 | 40754 | 41007 | 41681 |
| -4 | 7 | -299 | -150 | -287 | -287 | -275 | -284 | -275 | -223 | -278 | -235 | -204 | -238 | -217 | -37 | -122 |
| -5 | 1 | -323 | -162 | -308 | -299 | -317 | -262 | -171 | -308 | -314 | -302 | -305 | -143 | -314 | -317 | -149 |
| -5 | 2 | -348 | -348 | -332 | -338 | -290 | -335 | -180 | -290 | -311 | -332 | -332 | -329 | -274 | -192 | -256 |
| -5 | 3 | -281 | -277 | -204 | -262 | -271 | -262 | -250 | -198 | -265 | -265 | -125 | -253 | -122 | -201 | -232 |
| -5 | 4 | -244 | -336 | -320 | -308 | -183 | -217 | -302 | -207 | -241 | -317 | -302 | -250 | -207 | -314 | -149 |
| -5 | 5 | -312 | -257 | -308 | -302 | -293 | -171 | -296 | -241 | -153 | -287 | -180 | -196 | -305 | -238 | -232 |
| -5 | 6 | -293 | -235 | -189 | -289 | -293 | -262 | -134 | -192 | -231 | -174 | -250 | -177 | -283 | -262 | -259 |
| -5 | 7 | -360 | -376 | -391 | -299 | -330 | -327 | -379 | -220 | -376 | -266 | -229 | -367 | -373 | -342 | -302 |
| neg | 1 | -339 | -275 | -186 | -311 | -311 | -308 | -259 | -320 | -256 | -153 | -229 | -244 | -259 | -284 | -275 |
| neg | 2 | -299 | -296 | -244 | -281 | -128 | -290 | -269 | -272 | -183 | -174 | -269 | -256 | -110 | -131 | -262 |
| neg | 3 | -165 | -308 | -305 | -152 | -281 | -314 | -302 | -152 | -265 | -265 | -281 | -274 | -213 | -186 | -277 |
| neg | 4 | -250 | -338 | -335 | -250 | -326 | -323 | -323 | -277 | -320 | -289 | -177 | -323 | -302 | -280 | -305 |
| neg | 5 | -354 | -360 | -360 | -348 | -366 | -269 | -366 | -351 | -360 | -348 | -330 | -351 | -351 | -351 | -198 |
| neg | 6 | -421 | -379 | -296 | -427 | -415 | -275 | -415 | -421 | -418 | -418 | -421 | -409 | -415 | -412 | -308 |

**Appendix 5.6 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with raw sewage sample over 18 h.**

**4-MU-GAL**

| Dilution | Replicate | 17.5 | 18 |
|---|---|---|---|
| neat | 1 | 34244 | 34412 |
| neat | 2 | 46024 | 46757 |
| neat | 3 | 45598 | 45646 |
| neat | 4 | 44569 | 44807 |
| neat | 5 | 45350 | 45628 |
| neat | 6 | 44499 | 44527 |
| neat | 7 | 43577 | 43619 |
| -1 | 1 | 45100 | 45512 |
| -1 | 2 | 44236 | 44907 |
| -1 | 3 | 43891 | 44075 |
| -1 | 4 | 45167 | 45243 |
| -1 | 5 | 42005 | 42192 |
| -1 | 6 | 42937 | 42873 |
| -1 | 7 | 43650 | 43769 |
| -2 | 1 | 34712 | 37379 |
| -2 | 2 | 42185 | 42707 |
| -2 | 3 | 40501 | 40873 |
| -2 | 4 | 41844 | 42091 |
| -2 | 5 | 40351 | 41005 |
| -2 | 6 | 42229 | 42268 |
| -2 | 7 | 39790 | 40513 |
| -3 | 1 | 39683 | 40797 |
| -3 | 2 | -336 | -323 |
| -3 | 3 | 29618 | 33048 |
| -3 | 4 | 595 | 959 |
| -3 | 5 | -171 | -251 |
| -3 | 6 | -257 | -299 |
| -3 | 7 | 29954 | 31916 |
| -4 | 1 | -37 | 82 |
| -4 | 2 | -369 | -363 |
| -4 | 3 | -195 | -259 |
| -4 | 4 | -391 | -394 |
| -4 | 5 | -128 | -287 |
| -4 | 6 | 41846 | 41901 |
| -4 | 7 | 15 | 171 |
| -5 | 1 | -229 | -296 |
| -5 | 2 | -326 | -320 |
| -5 | 3 | -107 | -244 |
| -5 | 4 | -299 | -177 |
| -5 | 5 | -299 | -290 |
| -5 | 6 | -271 | -283 |
| -5 | 7 | -244 | -318 |
| neg | 1 | -177 | -299 |
| neg | 2 | -198 | -265 |
| neg | 3 | -250 | -271 |
| neg | 4 | -311 | -320 |
| neg | 5 | -354 | -351 |
| neg | 6 | -409 | -406 |

## Appendix 5.7: Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with settled sewage sample over 18 h.

EHC-GAL

| Dilution | Replicate | 0 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 0 | 1825 | 1822 | 2197 | 2417 | 2594 | 2887 | 3274 | 3808 | 4931 | 6460 | 8835 | 12268 | 17224 | 22824 |
| neat | 2 | 0 | 1645 | 1731 | 2005 | 2195 | 2420 | 2634 | 2970 | 3244 | 3660 | 4639 | 6791 | 10123 | 15101 | 20799 |
| neat | 3 | 0 | 1788 | 1904 | 2274 | 2381 | 2707 | 2924 | 3211 | 3659 | 4737 | 6940 | 10791 | 16276 | 23310 | 30296 |
| neat | 4 | 0 | 1462 | 1501 | 1770 | 1877 | 2115 | 2335 | 2634 | 2811 | 3186 | 4077 | 6143 | 9216 | 13782 | 19281 |
| neat | 5 | 0 | 1300 | 1599 | 1657 | 1876 | 2148 | 2475 | 2591 | 2957 | 3930 | 5813 | 9234 | 14600 | 21130 | 28153 |
| neat | 6 | 0 | 1480 | 1682 | 1856 | 2057 | 2493 | 2536 | 2841 | 3070 | 3491 | 4398 | 6131 | 8753 | 12702 | 17460 |
| neat | 7 | 0 | 1627 | 1789 | 1938 | 2253 | 2488 | 2622 | 3025 | 3220 | 3800 | 4889 | 7282 | 10908 | 15848 | 21812 |
| -1 | 1 | 0 | -174 | -204 | -101 | -119 | -162 | -150 | -125 | -89 | 49 | 64 | 256 | 742 | 1889 | 4453 |
| -1 | 2 | 0 | -330 | -379 | -287 | -339 | -345 | -367 | -214 | -251 | -315 | -296 | -119 | -257 | -119 | 27 |
| -1 | 3 | 0 | -131 | -128 | -18 | -116 | -76 | -73 | 24 | 125 | 39 | 235 | 708 | 1626 | 3491 | 6226 |
| -1 | 4 | 0 | -113 | -113 | -15 | -107 | -64 | -49 | 98 | -43 | -37 | 0 | 91 | 281 | 735 | 1978 |
| -1 | 5 | 0 | -125 | 27 | -119 | -101 | -67 | 73 | -64 | -34 | 27 | 12 | 52 | 156 | 247 | 461 |
| -1 | 6 | 0 | -183 | -134 | -177 | -155 | -115 | -119 | 46 | -61 | -54 | 4 | 284 | 541 | 1450 | 2991 |
| -1 | 7 | 0 | -39 | -24 | -42 | 3 | 16 | -24 | 70 | 186 | 58 | 177 | 232 | 263 | 577 | 815 |
| -2 | 1 | 0 | -231 | -247 | -219 | -253 | -244 | -296 | -311 | -149 | -283 | -296 | -262 | -119 | -15 | 245 |
| -2 | 2 | 0 | -150 | -229 | -232 | -232 | -238 | -278 | -260 | -119 | -202 | -119 | 79 | 689 | 1910 | 3772 |
| -2 | 3 | 0 | -122 | -189 | -225 | -308 | -317 | -326 | -317 | -305 | -351 | -225 | -250 | -290 | -161 | 19 |
| -2 | 4 | 0 | -326 | -335 | -232 | -366 | -342 | -348 | -403 | -396 | -418 | -424 | -424 | -430 | -409 | -287 |
| -2 | 5 | 0 | -103 | -152 | -311 | -320 | -317 | -192 | -357 | -271 | -348 | -366 | -372 | -348 | -369 | -369 |
| -2 | 6 | 0 | -315 | -232 | -330 | -354 | -360 | -366 | -415 | -342 | -360 | -348 | -287 | 39 | 522 | 1691 |
| -2 | 7 | 0 | -183 | -170 | -228 | -204 | -213 | -244 | -228 | -177 | -170 | -122 | -228 | -231 | -85 | -228 |
| -3 | 1 | 0 | -232 | -265 | -256 | -275 | -140 | -311 | -305 | -223 | -333 | -339 | -201 | -183 | -333 | -323 |
| -3 | 2 | 0 | -144 | -293 | -318 | -321 | -315 | -363 | -382 | -275 | -257 | -247 | -382 | -372 | -253 | -195 |
| -3 | 3 | 0 | -250 | -253 | -409 | -415 | -445 | -473 | -482 | -476 | -354 | -479 | -497 | -418 | -515 | -402 |
| -3 | 4 | 0 | -427 | -437 | -321 | -488 | -433 | -482 | -525 | -522 | -531 | -534 | -549 | -552 | -525 | -412 |
| -3 | 5 | 0 | -73 | -146 | -277 | -302 | -311 | -213 | -348 | -177 | -360 | -372 | -274 | -363 | -384 | -381 |
| -3 | 6 | 0 | -275 | -162 | -315 | -351 | -333 | -361 | -382 | -232 | -278 | -345 | -422 | -263 | -434 | -419 |
| -3 | 7 | 0 | -262 | -262 | -314 | -323 | -338 | -354 | -369 | -341 | -253 | -323 | -387 | -402 | -308 | -433 |
| -4 | 1 | 0 | -281 | -305 | -308 | -336 | -220 | -278 | -247 | -345 | -400 | -238 | -311 | -272 | -403 | -412 |
| -4 | 2 | 0 | -159 | -314 | -330 | -360 | -314 | -397 | -397 | -409 | -308 | -394 | -430 | -427 | -339 | -376 |
| -4 | 3 | 0 | -290 | -287 | -397 | -269 | -296 | -452 | -473 | -467 | -422 | -492 | -501 | -336 | -501 | -379 |
| -4 | 4 | 0 | -388 | -385 | -309 | -458 | -382 | -412 | -480 | -486 | -467 | -495 | -504 | -535 | -477 | -400 |
| -4 | 5 | 0 | -110 | -192 | -259 | -305 | -314 | -235 | -201 | -247 | -357 | -204 | -137 | 15 | 507 | 1367 |
| -4 | 6 | 0 | -266 | -183 | -269 | -336 | -321 | -342 | -354 | -324 | -333 | -229 | -388 | -269 | -415 | -397 |
| -4 | 7 | 0 | -373 | -388 | -434 | -431 | -443 | -431 | -486 | -474 | -440 | -480 | -516 | -519 | -455 | -550 |
| -5 | 1 | 0 | -232 | -134 | -272 | -278 | -232 | -159 | -275 | -317 | -281 | -266 | -305 | -263 | -339 | -339 |
| -5 | 2 | 0 | -214 | -312 | -345 | -360 | -266 | -406 | -391 | -409 | -434 | -458 | -421 | -425 | -306 | -412 |
| -5 | 3 | 0 | -278 | -263 | -354 | -260 | -229 | -409 | -403 | -422 | -412 | -446 | -446 | -339 | -455 | -403 |
| -5 | 4 | 0 | -247 | -235 | -259 | -314 | -213 | -268 | -320 | -311 | -284 | -317 | -290 | -384 | -302 | -409 |
| -5 | 5 | 0 | -150 | -248 | -260 | -330 | -345 | -260 | -260 | -336 | -406 | -269 | -385 | -418 | -425 | -425 |
| -5 | 6 | 0 | -217 | -263 | -208 | -314 | -278 | -308 | -250 | -366 | -369 | -409 | -339 | -388 | -391 | -327 |
| -5 | 7 | 0 | -305 | -324 | -363 | -376 | -379 | -379 | -421 | -424 | -397 | -431 | -431 | -464 | -418 | -489 |
| neg | 1 | 0 | -202 | -186 | -263 | -269 | -131 | -235 | -229 | -244 | -171 | -217 | -241 | -333 | -238 | -379 |
| neg | 2 | 0 | -161 | -158 | -271 | -271 | -180 | -210 | -195 | -165 | -338 | -226 | -335 | -311 | -201 | -375 |
| neg | 3 | 0 | -126 | -150 | -287 | -266 | -275 | -199 | -342 | -269 | -364 | -358 | -379 | -309 | -239 | -391 |
| neg | 4 | 0 | -128 | -155 | -342 | -323 | -354 | -241 | -403 | -406 | -421 | -436 | -415 | -348 | -421 | -454 |
| neg | 5 | 0 | -363 | -281 | -437 | -342 | -461 | -324 | -485 | -501 | -485 | -519 | -461 | -382 | -534 | -507 |
| neg | 6 | 0 | -289 | -317 | -329 | -204 | -363 | -393 | -347 | -399 | -360 | -399 | -311 | -442 | -457 | -326 |

**Appendix 5.7 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with settled sewage sample over 18 h.**

EHC-GAL

| Dilution | Replicate | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 | 14 | 14.5 | 15 | 15.5 | 16 | 16.5 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 28861 | 35331 | 41517 | 47242 | 50849 | 52967 | 53486 | 53773 | 54417 | 54276 | 54374 | 54072 | 53959 | 54487 | 54322 |
| neat | 2 | 26744 | 31935 | 37242 | 42283 | 45528 | 48241 | 49919 | 51125 | 51393 | 51839 | 52202 | 52586 | 52397 | 52397 | 52013 |
| neat | 3 | 38078 | 43550 | 47270 | 49138 | 50490 | 51744 | 51814 | 52824 | 52867 | 52617 | 52730 | 52666 | 52681 | 53047 | 53026 |
| neat | 4 | 24961 | 30872 | 37333 | 43690 | 47044 | 48918 | 49848 | 50981 | 50489 | 51176 | 50880 | 51197 | 50819 | 50880 | 50804 |
| neat | 5 | 34888 | 40760 | 45869 | 48792 | 50654 | 52207 | 52967 | 53486 | 53730 | 53669 | 53599 | 53959 | 53840 | 53721 | 53663 |
| neat | 6 | 23340 | 29887 | 36103 | 41914 | 46254 | 48793 | 50953 | 52046 | 52519 | 52858 | 52678 | 52772 | 52748 | 52824 | 52583 |
| neat | 7 | 27918 | 33601 | 39335 | 43553 | 47011 | 49217 | 50731 | 51768 | 51982 | 51982 | 52757 | 53041 | 53011 | 52898 | 52965 |
| -1 | 1 | 8695 | 14301 | 20743 | 27308 | 33118 | 38954 | 44017 | 47477 | 50517 | 51997 | 52867 | 53346 | 53697 | 54246 | 54118 |
| -1 | 2 | 262 | 415 | 906 | 1739 | 2505 | 3695 | 5139 | 7168 | 9711 | 12686 | 15979 | 19785 | 23883 | 28073 | 32123 |
| -1 | 3 | 10312 | 15228 | 20850 | 27189 | 33546 | 39979 | 46565 | 50334 | 51872 | 52470 | 53031 | 53276 | 53285 | 53782 | 53428 |
| -1 | 4 | 4684 | 10620 | 29484 | 41364 | 46098 | 49205 | 50691 | 51750 | 51384 | 51829 | 51655 | 51985 | 51527 | 51567 | 51439 |
| -1 | 5 | 1135 | 2042 | 3922 | 6687 | 11218 | 17224 | 24943 | 32874 | 39521 | 44309 | 48118 | 50611 | 51997 | 53334 | 53511 |
| -1 | 6 | 5909 | 10041 | 15086 | 21009 | 26893 | 33195 | 39754 | 46205 | 49883 | 51869 | 52178 | 52959 | 53118 | 53459 | 53328 |
| -1 | 7 | 1612 | 3080 | 5503 | 9006 | 13221 | 17902 | 22846 | 27613 | 31440 | 35264 | 38496 | 41377 | 43641 | 45494 | 47441 |
| -2 | 1 | 574 | 1111 | 1908 | 3437 | 5674 | 9556 | 16618 | 24464 | 31654 | 38899 | 43642 | 47285 | 49968 | 51653 | 52306 |
| -2 | 2 | 6415 | 9848 | 14221 | 18774 | 23767 | 28705 | 33558 | 39948 | 46436 | 50227 | 52082 | 52445 | 52885 | 53712 | 53559 |
| -2 | 3 | 379 | 995 | 2198 | 4474 | 8127 | 13569 | 20359 | 27455 | 33339 | 38673 | 42778 | 45833 | 47801 | 49757 | 50377 |
| -2 | 4 | -409 | -378 | -277 | 25 | 1093 | 4825 | 12784 | 23402 | 33802 | 41859 | 47023 | 49925 | 50200 | 50758 | 50764 |
| -2 | 5 | -342 | -381 | -369 | -366 | -360 | -366 | -375 | -363 | -375 | -381 | -262 | -338 | -378 | -238 | -387 |
| -2 | 6 | 4153 | 9549 | 23004 | 33961 | 40495 | 45948 | 49296 | 51814 | 52778 | 53099 | 53178 | 53752 | 53758 | 54002 | 53852 |
| -2 | 7 | -207 | -15 | 80 | 214 | 379 | 464 | 800 | 1273 | 1694 | 2414 | 3052 | 3928 | 4899 | 6122 | 7236 |
| -3 | 1 | -351 | -342 | -391 | -293 | -326 | -375 | -360 | -174 | -210 | -46 | 259 | 733 | 1474 | 2185 | 3287 |
| -3 | 2 | -278 | -195 | 36 | 79 | 278 | 638 | 1028 | 1642 | 2545 | 3668 | 5176 | 6989 | 9128 | 12009 | 15048 |
| -3 | 3 | -543 | -552 | -402 | -528 | -582 | -570 | -573 | -564 | -405 | -512 | -436 | -479 | -470 | -582 | -470 |
| -3 | 4 | -562 | -580 | -552 | -592 | -556 | -620 | -629 | -598 | -620 | -629 | -635 | -601 | -656 | -659 | -659 |
| -3 | 5 | -372 | -418 | -418 | -299 | -439 | -430 | -445 | -439 | -442 | -341 | -378 | -430 | -470 | -406 | -338 |
| -3 | 6 | -422 | -318 | -287 | -287 | -452 | -431 | -452 | -370 | -394 | -394 | -345 | -257 | -110 | 216 | 735 |
| -3 | 7 | -421 | -299 | -381 | -366 | -418 | -485 | -491 | -469 | -347 | -473 | -515 | -512 | -512 | -363 | -543 |
| -4 | 1 | -443 | -436 | -452 | -433 | -458 | -348 | -482 | -400 | -336 | -366 | -522 | -366 | -443 | -534 | -473 |
| -4 | 2 | -473 | -437 | -476 | -491 | -519 | -376 | -507 | -522 | -443 | -537 | -421 | -540 | -595 | -537 | -485 |
| -4 | 3 | -489 | -553 | -498 | -547 | -397 | -510 | -580 | -571 | -513 | -592 | -544 | -538 | -553 | -629 | -522 |
| -4 | 4 | -498 | -559 | -416 | -580 | -443 | -583 | -553 | -623 | -605 | -629 | -632 | -574 | -660 | -660 | -638 |
| -4 | 5 | 2725 | 4614 | 7251 | 11069 | 15265 | 21757 | 31718 | 41627 | 48216 | 51802 | 53499 | 54338 | 54860 | 55519 | 55769 |
| -4 | 6 | -437 | -433 | -449 | -464 | -403 | -406 | -452 | -330 | -427 | -507 | -516 | -510 | -516 | -433 | -449 |
| -4 | 7 | -391 | -480 | -565 | -528 | -583 | -449 | -620 | -593 | -535 | -632 | -644 | -635 | -651 | -577 | -538 |
| -5 | 1 | -354 | -354 | -259 | -366 | -391 | -256 | -272 | -366 | -321 | -333 | -433 | -293 | -409 | -452 | -452 |
| -5 | 2 | -443 | -397 | -470 | -486 | -479 | -357 | -418 | -507 | -345 | -516 | -376 | -531 | -547 | -544 | -434 |
| -5 | 3 | -348 | -480 | -461 | -477 | -446 | -394 | -348 | -455 | -489 | -535 | -489 | -498 | -516 | -562 | -531 |
| -5 | 4 | -284 | -403 | -403 | -442 | -451 | -390 | -332 | -482 | -403 | -470 | -494 | -427 | -516 | -519 | -430 |
| -5 | 5 | -455 | -455 | -382 | -360 | -367 | -342 | -333 | -495 | -345 | -476 | -495 | -510 | -537 | -516 | -501 |
| -5 | 6 | -403 | -437 | -430 | -452 | -354 | -333 | -357 | -424 | -318 | -470 | -491 | -479 | -485 | -397 | -379 |
| -5 | 7 | -363 | -421 | -504 | -495 | -531 | -437 | -418 | -550 | -528 | -574 | -589 | -580 | -586 | -525 | -519 |
| neg | 1 | -308 | -366 | -400 | -400 | -424 | -263 | -318 | -440 | -284 | -382 | -443 | -369 | -473 | -455 | -333 |
| neg | 2 | -369 | -326 | -393 | -387 | -384 | -421 | -421 | -418 | -436 | -277 | -433 | -357 | -464 | -418 | -470 |
| neg | 3 | -397 | -315 | -412 | -391 | -306 | -449 | -458 | -443 | -477 | -461 | -449 | -333 | -492 | -379 | -522 |
| neg | 4 | -445 | -329 | -421 | -427 | -503 | -519 | -519 | -491 | -537 | -555 | -482 | -390 | -561 | -436 | -592 |
| neg | 5 | -510 | -449 | -455 | -437 | -604 | -589 | -559 | -540 | -623 | -638 | -531 | -565 | -650 | -672 | -659 |
| neg | 6 | -335 | -482 | -488 | -503 | -470 | -448 | -372 | -381 | -473 | -500 | -445 | -570 | -579 | -585 | -491 |

**Appendix 5.7 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with settled sewage sample over 18 h.**

EHC-GAL

| Dilution | Replicate | 17.5 | 18 |
|---|---|---|---|
| neat | 1 | 54307 | 54521 |
| neat | 2 | 52669 | 52013 |
| neat | 3 | 52919 | 53093 |
| neat | 4 | 51246 | 50941 |
| neat | 5 | 54148 | 54130 |
| neat | 6 | 53074 | 52922 |
| neat | 7 | 53407 | 53325 |
| -1 | 1 | 54262 | 54439 |
| -1 | 2 | 37150 | 40708 |
| -1 | 3 | 53343 | 53556 |
| -1 | 4 | 52012 | 51677 |
| -1 | 5 | 54442 | 54619 |
| -1 | 6 | 53780 | 53243 |
| -1 | 7 | 49458 | 50310 |
| -2 | 1 | 53234 | 53923 |
| -2 | 2 | 53425 | 53608 |
| -2 | 3 | 51353 | 51991 |
| -2 | 4 | 51421 | 51076 |
| -2 | 5 | -375 | -381 |
| -2 | 6 | 54280 | 53556 |
| -2 | 7 | 8713 | 10187 |
| -3 | 1 | 4514 | 5683 |
| -3 | 2 | 18543 | 22589 |
| -3 | 3 | -485 | -628 |
| -3 | 4 | -604 | -662 |
| -3 | 5 | -488 | -497 |
| -3 | 6 | 1721 | 3192 |
| -3 | 7 | -540 | -567 |
| -4 | 1 | -415 | -565 |
| -4 | 2 | -592 | -546 |
| -4 | 3 | -483 | -541 |
| -4 | 4 | -556 | -635 |
| -4 | 5 | 55549 | 55440 |
| -4 | 6 | -473 | -549 |
| -4 | 7 | -684 | -702 |
| -5 | 1 | -412 | -339 |
| -5 | 2 | -562 | -467 |
| -5 | 3 | -467 | -455 |
| -5 | 4 | -378 | -445 |
| -5 | 5 | -559 | -571 |
| -5 | 6 | -397 | -522 |
| -5 | 7 | -614 | -635 |
| neg | 1 | -345 | -357 |
| neg | 2 | -470 | -378 |
| neg | 3 | -528 | -538 |
| neg | 4 | -583 | -604 |
| neg | 5 | -696 | -708 |
| neg | 6 | -598 | -592 |

**Appendix 5.7 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with settled sewage sample over 18 h.**

4-MU-GAL

| Dilution | Replicate | 0 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 0 | 17304 | 17331 | 17337 | 17325 | 17328 | 17371 | 17356 | 17362 | 17298 | 17191 | 17075 | 17639 | 19825 | 22629 |
| neat | 2 | 0 | 1206 | 1435 | 1728 | 1822 | 2063 | 2246 | 2463 | 2909 | 3263 | 3852 | 5234 | 7740 | 11289 | 15848 |
| neat | 3 | 0 | 934 | 1135 | 1404 | 1566 | 1837 | 1987 | 2249 | 2481 | 3015 | 4117 | 5982 | 8976 | 13138 | 17801 |
| neat | 4 | 0 | 1196 | 1361 | 1693 | 1812 | 2185 | 2234 | 2560 | 2856 | 3170 | 3900 | 5118 | 7150 | 10327 | 14886 |
| neat | 5 | 0 | 1232 | 1379 | 1590 | 1809 | 2020 | 2252 | 2533 | 3033 | 3640 | 4925 | 7193 | 10388 | 15079 | 20413 |
| neat | 6 | 0 | 1032 | 1181 | 1346 | 1535 | 1728 | 1886 | 2097 | 2372 | 2805 | 3366 | 4541 | 7126 | 11222 | 16605 |
| neat | 7 | 0 | 1254 | 1382 | 1514 | 1755 | 1944 | 2121 | 2387 | 2680 | 2951 | 3546 | 4822 | 7083 | 10706 | 15439 |
| -1 | 1 | 0 | -15 | -134 | -116 | -94 | -76 | 110 | 68 | 83 | 132 | 98 | 318 | 382 | 681 | 1325 |
| -1 | 2 | 0 | -131 | -73 | 12 | -107 | -76 | -49 | -24 | 52 | 52 | 168 | 382 | 821 | 1230 | 2112 |
| -1 | 3 | 0 | 64 | 86 | 147 | 189 | 388 | 284 | 434 | 379 | 430 | 495 | 705 | 647 | 922 | 1016 |
| -1 | 4 | 0 | -82 | -113 | 49 | -97 | -27 | -55 | -24 | 3 | 104 | 89 | 193 | 397 | 809 | 1801 |
| -1 | 5 | 0 | -67 | -43 | -37 | 3 | 21 | 64 | 94 | 265 | 238 | 336 | 650 | 824 | 1382 | 2524 |
| -1 | 6 | 0 | -40 | -55 | -43 | -21 | -18 | -3 | 186 | 61 | 134 | 150 | 351 | 968 | 1938 | 4123 |
| -1 | 7 | 0 | -24 | 4 | -76 | -21 | -12 | -6 | 98 | 196 | 95 | 89 | 150 | 245 | 550 | 1136 |
| -2 | 1 | 0 | -183 | -125 | -210 | -207 | -64 | -162 | -207 | -207 | -195 | -94 | -183 | -186 | -49 | -183 |
| -2 | 2 | 0 | -125 | -76 | -34 | -177 | -171 | -168 | -131 | -156 | -147 | -143 | -137 | 55 | 43 | 540 |
| -2 | 3 | 0 | -149 | -168 | -162 | -165 | -55 | -149 | -119 | -146 | -131 | -131 | -76 | -104 | -15 | 22 |
| -2 | 4 | 0 | -79 | -144 | -15 | -144 | -134 | -137 | -134 | -113 | 49 | -43 | 73 | 119 | 400 | 894 |
| -2 | 5 | 0 | -278 | -265 | -268 | -259 | -253 | -253 | -265 | -128 | -220 | -256 | -241 | -238 | -232 | -235 |
| -2 | 6 | 0 | -128 | 13 | -106 | -128 | -137 | -131 | -51 | -116 | -85 | -106 | 58 | 40 | -88 | -30 |
| -2 | 7 | 0 | -268 | -143 | -284 | -250 | -235 | -268 | -128 | -220 | -171 | -256 | -171 | -253 | -195 | -232 |
| -3 | 1 | 0 | -180 | -183 | -83 | -37 | -171 | -202 | -186 | -165 | -189 | -147 | -193 | -119 | -125 | -34 |
| -3 | 2 | 0 | -67 | -180 | -183 | -195 | -192 | -201 | -100 | -195 | -201 | -180 | -180 | -55 | -180 | -46 |
| -3 | 3 | 0 | -37 | -180 | -171 | -177 | -116 | -168 | -177 | -165 | -174 | -174 | -146 | -165 | -134 | -165 |
| -3 | 4 | 0 | -104 | -171 | -52 | -177 | -183 | -174 | -183 | -180 | -122 | -107 | -34 | -168 | -168 | -150 |
| -3 | 5 | 0 | -297 | -293 | -293 | -284 | -278 | -284 | -297 | -297 | -226 | -312 | -300 | -300 | -290 | -297 |
| -3 | 6 | 0 | -168 | -67 | -143 | -113 | -186 | -192 | -161 | -195 | -183 | -186 | -85 | -82 | -174 | -152 |
| -3 | 7 | 0 | -192 | -64 | -201 | -149 | -152 | -216 | -192 | -204 | -88 | -155 | -52 | -201 | -85 | -207 |
| -4 | 1 | 0 | -318 | -364 | -281 | -324 | -364 | -367 | -272 | -290 | -272 | -373 | -202 | -275 | -348 | -299 |
| -4 | 2 | 0 | -40 | -210 | -204 | -204 | -217 | -213 | -64 | -210 | -217 | -155 | -186 | -61 | -201 | -192 |
| -4 | 3 | 0 | -28 | -186 | -198 | -198 | -174 | -208 | -198 | -40 | -180 | -183 | -189 | -180 | -177 | -174 |
| -4 | 4 | 0 | -80 | -156 | -180 | -199 | -208 | -214 | -211 | -199 | -205 | -49 | -186 | -196 | -192 | -134 |
| -4 | 5 | 0 | -199 | -202 | -162 | -177 | -150 | -174 | -156 | -214 | -49 | -192 | -211 | -199 | -180 | -171 |
| -4 | 6 | 0 | -235 | -94 | -106 | -125 | -256 | -253 | -250 | -253 | -256 | -256 | -219 | -177 | -247 | -229 |
| -4 | 7 | 0 | -186 | -73 | -192 | -113 | -46 | -207 | -217 | -217 | -204 | -58 | -201 | -192 | -61 | -204 |
| -5 | 1 | 0 | -89 | -214 | -208 | -220 | -223 | -223 | -226 | -220 | -214 | -226 | -186 | -201 | -223 | -217 |
| -5 | 2 | 0 | -34 | -168 | -177 | -180 | -177 | -180 | -141 | -156 | -183 | -37 | -98 | -67 | -168 | -165 |
| -5 | 3 | 0 | -232 | -323 | -342 | -345 | -335 | -348 | -342 | -259 | -186 | -180 | -345 | -320 | -326 | -192 |
| -5 | 4 | 0 | -34 | -147 | -193 | -186 | -205 | -205 | -196 | -171 | -211 | -186 | -199 | -193 | -174 | -86 |
| -5 | 5 | 0 | -162 | -195 | -94 | -113 | -67 | -113 | -46 | -198 | -183 | -110 | -195 | -198 | -122 | -125 |
| -5 | 6 | 0 | -214 | -74 | -52 | -156 | -229 | -242 | -226 | -223 | -232 | -226 | -220 | -156 | -205 | -199 |
| -5 | 7 | 0 | -174 | -165 | -159 | -68 | -129 | -177 | -193 | -193 | -193 | -187 | -196 | -147 | -135 | -196 |
| neg | 1 | 0 | -162 | -101 | -205 | -220 | -144 | -119 | -223 | -217 | -223 | -64 | -214 | -208 | -202 | -205 |
| neg | 2 | 0 | -204 | -73 | -226 | -232 | -98 | -98 | -226 | -217 | -232 | -168 | -229 | -217 | -217 | -211 |
| neg | 3 | 0 | -341 | -259 | -357 | -350 | -286 | -274 | -192 | -216 | -335 | -363 | -341 | -344 | -262 | -228 |
| neg | 4 | 0 | -241 | -189 | -250 | -250 | -229 | -183 | -171 | -165 | -100 | -259 | -110 | -155 | -119 | -152 |
| neg | 5 | 0 | -232 | -214 | -241 | -183 | -235 | -217 | -238 | -241 | -162 | -254 | -202 | -104 | -110 | -202 |
| neg | 6 | 0 | -193 | -199 | -129 | -89 | -235 | -202 | -238 | -229 | -199 | -232 | -214 | -122 | -110 | -220 |

**Appendix 5.7 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with settled sewage sample over 18 h.**

**4-MU-GAL**

| Dilution | Replicate | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 | 14 | 14.5 | 15 | 15.5 | 16 | 16.5 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 25312 | 28339 | 30836 | 32957 | 33872 | 34092 | 34092 | 34074 | 34037 | 34031 | 33994 | 33973 | 33964 | 33958 | 33994 |
| neat | 2 | 20829 | 26042 | 30329 | 34019 | 37013 | 38963 | 40706 | 41710 | 42112 | 42277 | 42598 | 43159 | 43437 | 43907 | 44295 |
| neat | 3 | 22584 | 27811 | 31934 | 35822 | 37840 | 39811 | 40895 | 41325 | 41682 | 42244 | 42726 | 43116 | 43342 | 44038 | 44490 |
| neat | 4 | 21222 | 28891 | 34299 | 37317 | 38999 | 39728 | 40595 | 40867 | 41544 | 41547 | 41785 | 42213 | 43101 | 43564 | 43519 |
| neat | 5 | 26190 | 31623 | 35608 | 38584 | 40268 | 41318 | 41492 | 42038 | 42322 | 42728 | 43311 | 43708 | 43668 | 43815 | 44202 |
| neat | 6 | 21903 | 27155 | 31895 | 35325 | 38121 | 39723 | 40895 | 41578 | 41801 | 42259 | 42732 | 42906 | 43416 | 44292 | 44212 |
| neat | 7 | 21000 | 26774 | 32758 | 36268 | 38151 | 39112 | 39915 | 40376 | 40638 | 40702 | 41307 | 41346 | 41954 | 42048 | 42152 |
| -1 | 1 | 2436 | 4075 | 6476 | 9507 | 12965 | 17197 | 22358 | 27897 | 32991 | 36934 | 39546 | 41414 | 42558 | 43181 | 43285 |
| -1 | 2 | 3162 | 4959 | 7230 | 10306 | 14042 | 17951 | 22309 | 26292 | 29810 | 32606 | 34718 | 36531 | 37516 | 38346 | 39012 |
| -1 | 3 | 1477 | 1883 | 2722 | 3678 | 5161 | 7025 | 9848 | 13782 | 18470 | 23359 | 28877 | 33509 | 37123 | 39839 | 41587 |
| -1 | 4 | 3696 | 6592 | 10477 | 15189 | 19993 | 25047 | 29173 | 32591 | 34669 | 36314 | 36976 | 37285 | 38255 | 38774 | 39094 |
| -1 | 5 | 4739 | 8356 | 13526 | 21195 | 28772 | 34785 | 37254 | 38087 | 38548 | 38996 | 39631 | 40080 | 39970 | 40257 | 40772 |
| -1 | 6 | 7041 | 10999 | 15986 | 21360 | 27186 | 32096 | 35838 | 37998 | 39009 | 39061 | 39421 | 39564 | 39845 | 40538 | 40782 |
| -1 | 7 | 1880 | 2820 | 4526 | 6791 | 10368 | 15223 | 20823 | 26973 | 31721 | 35319 | 37651 | 38493 | 39323 | 39607 | 39854 |
| -2 | 1 | -155 | -159 | -162 | -152 | -140 | -67 | -67 | -94 | -94 | -110 | 3 | -6 | -3 | -30 | -24 |
| -2 | 2 | 1022 | 2142 | 3610 | 5557 | 8017 | 10571 | 13828 | 17334 | 21442 | 25641 | 30027 | 34928 | 38053 | 39112 | 39320 |
| -2 | 3 | 363 | 678 | 1407 | 2460 | 3992 | 5881 | 8323 | 11338 | 14655 | 18275 | 22050 | 25544 | 29524 | 32813 | 35337 |
| -2 | 4 | 1620 | 2634 | 4016 | 6030 | 8939 | 14170 | 21076 | 28571 | 34663 | 39381 | 41535 | 42124 | 43019 | 43202 | 43513 |
| -2 | 5 | -85 | -33 | -156 | 6 | -21 | 110 | 314 | 522 | 873 | 1349 | 2048 | 2918 | 3751 | 4969 | 6525 |
| -2 | 6 | 31 | 25 | -45 | 68 | -33 | 104 | 13 | 58 | 150 | 312 | 318 | 657 | 953 | 1322 | 2015 |
| -2 | 7 | -232 | -232 | -107 | -220 | -204 | -183 | -156 | 110 | 205 | 684 | 1373 | 2231 | 3824 | 6729 | 12146 |
| -3 | 1 | -180 | -144 | -168 | -171 | -165 | -177 | -165 | -165 | -171 | -19 | -156 | -141 | -31 | -150 | -150 |
| -3 | 2 | -189 | -119 | -174 | -180 | -183 | -174 | -46 | -76 | -79 | -158 | -165 | -134 | -12 | -52 | -116 |
| -3 | 3 | -125 | -153 | -150 | -153 | -156 | -9 | -134 | -95 | -131 | -46 | -119 | -131 | -110 | -107 | -104 |
| -3 | 4 | -165 | -159 | -165 | -58 | -150 | -22 | -156 | -125 | -134 | 9 | -125 | -113 | 30 | 58 | -52 |
| -3 | 5 | -290 | -275 | -293 | -284 | -300 | -290 | -181 | -281 | -242 | -266 | -181 | -199 | -235 | -260 | -101 |
| -3 | 6 | -128 | -33 | -152 | -70 | -45 | 113 | 80 | 199 | 360 | 550 | 690 | 1026 | 1291 | 1471 | 1804 |
| -3 | 7 | -201 | -189 | -198 | -186 | -64 | -79 | -198 | -122 | -204 | -79 | -91 | -201 | -73 | -186 | -177 |
| -4 | 1 | -199 | -217 | -211 | -223 | -223 | -351 | -260 | -354 | -284 | -324 | -351 | -345 | -290 | -339 | -339 |
| -4 | 2 | -198 | -116 | -165 | -192 | -204 | -189 | -198 | -189 | -49 | -122 | -195 | -162 | -104 | -49 | -183 |
| -4 | 3 | -180 | -177 | -177 | -180 | -177 | -119 | -31 | -144 | -159 | -159 | -162 | -174 | -156 | -98 | -165 |
| -4 | 4 | -189 | -199 | -202 | -37 | -150 | -153 | -186 | -165 | -186 | -113 | -196 | -183 | -58 | -156 | -31 |
| -4 | 5 | -199 | -196 | -199 | -211 | -180 | -196 | -107 | -205 | -58 | -95 | -64 | -202 | -202 | -192 | -202 |
| -4 | 6 | -238 | -106 | -253 | -216 | -134 | -195 | -100 | -241 | -253 | -238 | -235 | -88 | -131 | -241 | -235 |
| -4 | 7 | -201 | -168 | -214 | -125 | -192 | -67 | -159 | -168 | -165 | -110 | -6 | -159 | -152 | -140 | -61 |
| -5 | 1 | -174 | -174 | -189 | -159 | -211 | -58 | -168 | -201 | -125 | -214 | -208 | -214 | -201 | -125 | -49 |
| -5 | 2 | -153 | -76 | -76 | -128 | -131 | -107 | -153 | -141 | -141 | 6 | -131 | -64 | -55 | 3 | -141 |
| -5 | 3 | -317 | -317 | -302 | -305 | -171 | -293 | -219 | -290 | -296 | -299 | -302 | -290 | -290 | -162 | -287 |
| -5 | 4 | -159 | -177 | -159 | -153 | -16 | -171 | -116 | -119 | -135 | -153 | -150 | -159 | -3 | -147 | -22 |
| -5 | 5 | -201 | -183 | -162 | -204 | -58 | -180 | -195 | -183 | -180 | -97 | -189 | -189 | -189 | -168 | -183 |
| -5 | 6 | -205 | -74 | -181 | -126 | -86 | -40 | 94 | 100 | 253 | 466 | 802 | 1409 | 2081 | 2923 | 4400 |
| -5 | 7 | -159 | -104 | -193 | -34 | -174 | -190 | -65 | -177 | -153 | -181 | -89 | -168 | -171 | -123 | -58 |
| neg | 1 | -205 | -168 | -195 | -171 | -186 | -128 | -180 | -21 | -165 | -131 | -76 | -86 | -171 | -79 | -131 |
| neg | 2 | -217 | -195 | -217 | -208 | -113 | -198 | -189 | -76 | -189 | -116 | -76 | -46 | -189 | -150 | -180 |
| neg | 3 | -195 | -338 | -238 | -354 | -283 | -347 | -198 | -296 | -338 | -332 | -326 | -283 | -350 | -326 | -335 |
| neg | 4 | -186 | -244 | -113 | -241 | -253 | -247 | -192 | -198 | -128 | -250 | -241 | -226 | -259 | -250 | -250 |
| neg | 5 | -232 | -244 | -192 | -122 | -254 | -104 | -247 | -226 | -144 | -241 | -257 | -238 | -244 | -241 | -208 |
| neg | 6 | -232 | -229 | -211 | -83 | -232 | -168 | -241 | -214 | -171 | -107 | -229 | -217 | -232 | -226 | -86 |

**Appendix 5.7 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with settled sewage sample over 18 h.**

**4-MU-GAL**

| Dilution | Replicate | 17.5 | 18 |
|---|---|---|---|
| neat | 1 | 34098 | 34183 |
| neat | 2 | 44578 | 44453 |
| neat | 3 | 44899 | 44761 |
| neat | 4 | 43818 | 44529 |
| neat | 5 | 44312 | 44687 |
| neat | 6 | 44502 | 44514 |
| neat | 7 | 42982 | 42671 |
| -1 | 1 | 43901 | 44084 |
| -1 | 2 | 39289 | 39564 |
| -1 | 3 | 42421 | 42875 |
| -1 | 4 | 39509 | 40272 |
| -1 | 5 | 40849 | 41132 |
| -1 | 6 | 41023 | 40989 |
| -1 | 7 | 40663 | 40437 |
| -2 | 1 | 9 | 31 |
| -2 | 2 | 39213 | 39417 |
| -2 | 3 | 37126 | 38627 |
| -2 | 4 | 43598 | 43952 |
| -2 | 5 | 8237 | 10266 |
| -2 | 6 | 2988 | 4200 |
| -2 | 7 | 19880 | 28117 |
| -3 | 1 | -138 | -147 |
| -3 | 2 | -137 | -143 |
| -3 | 3 | -107 | -55 |
| -3 | 4 | -104 | -46 |
| -3 | 5 | -190 | -150 |
| -3 | 6 | 2219 | 2585 |
| -3 | 7 | -49 | -152 |
| -4 | 1 | -342 | -342 |
| -4 | 2 | -186 | -183 |
| -4 | 3 | -153 | -150 |
| -4 | 4 | -174 | -165 |
| -4 | 5 | -101 | -196 |
| -4 | 6 | -210 | -113 |
| -4 | 7 | -149 | -122 |
| -5 | 1 | -208 | -89 |
| -5 | 2 | -137 | -119 |
| -5 | 3 | -296 | -284 |
| -5 | 4 | -135 | -141 |
| -5 | 5 | -24 | -186 |
| -5 | 6 | 6698 | 10022 |
| -5 | 7 | -181 | -25 |
| neg | 1 | -25 | -89 |
| neg | 2 | -46 | -153 |
| neg | 3 | -302 | -332 |
| neg | 4 | -219 | -256 |
| neg | 5 | -232 | -241 |
| neg | 6 | -229 | -205 |

## Appendix 5.8: Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with filtered sewage sample over 18 h.

EHC-GAL

| Dilution | Replicate | 0 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 0 | -100 | -30 | -103 | 92 | 123 | 25 | 220 | 162 | 266 | 477 | 989 | 2463 | 5378 | 10068 |
| neat | 2 | 0 | 299 | 406 | 443 | 446 | 653 | 629 | 705 | 803 | 1050 | 1212 | 1834 | 3095 | 5481 | 9214 |
| neat | 3 | 0 | 1022 | 989 | 1166 | 1181 | 1358 | 1514 | 1630 | 1794 | 2017 | 2661 | 3799 | 6436 | 10870 | 16318 |
| neat | 4 | 0 | 506 | 531 | 668 | 784 | 876 | 1001 | 1126 | 1202 | 1361 | 1642 | 2084 | 2810 | 4645 | 8908 |
| neat | 5 | 0 | -351 | -305 | -363 | -369 | -296 | -375 | -278 | -241 | -250 | -278 | -140 | -9 | 351 | 1053 |
| neat | 6 | 0 | -448 | -366 | -430 | -344 | -354 | -439 | -442 | -323 | -412 | -286 | -320 | -51 | 620 | 1990 |
| neat | 7 | 0 | -30 | 9 | 18 | 67 | 128 | 208 | 211 | 256 | 324 | 446 | 778 | 1627 | 3058 | 5548 |
| -1 | 1 | 0 | -470 | -430 | -586 | -589 | -473 | -607 | -476 | -589 | -580 | -528 | -632 | -623 | -626 | -647 |
| -1 | 2 | 0 | -305 | -351 | -433 | -308 | -354 | -473 | -485 | -439 | -409 | -497 | -363 | -439 | -381 | -497 |
| -1 | 3 | 0 | -369 | -302 | -326 | -439 | -424 | -290 | -448 | -454 | -345 | -308 | -436 | -287 | -265 | -52 |
| -1 | 4 | 0 | -272 | -308 | -311 | -192 | -284 | -269 | -247 | -266 | -153 | -134 | 116 | 232 | 830 | 2017 |
| -1 | 5 | 0 | -400 | -339 | -461 | -513 | -461 | -543 | -434 | -388 | -427 | -550 | -482 | -550 | -525 | -501 |
| -1 | 6 | 0 | -378 | -534 | -403 | -540 | -512 | -580 | -528 | -518 | -589 | -470 | -604 | -561 | -528 | -592 |
| -1 | 7 | 0 | -379 | -315 | -394 | -415 | -397 | -397 | -421 | -424 | -434 | -443 | -406 | -412 | -327 | -443 |
| -2 | 1 | 0 | -363 | -561 | -561 | -573 | -461 | -589 | -491 | -570 | -561 | -512 | -622 | -616 | -598 | -635 |
| -2 | 2 | 0 | -341 | -424 | -454 | -424 | -363 | -467 | -485 | -457 | -424 | -506 | -396 | -457 | -387 | -512 |
| -2 | 3 | 0 | -327 | -251 | -440 | -446 | -434 | -339 | -464 | -477 | -391 | -361 | -495 | -351 | -477 | -510 |
| -2 | 4 | 0 | -433 | -442 | -463 | -436 | -451 | -433 | -454 | -488 | -405 | -454 | -329 | -424 | -286 | 113 |
| -2 | 5 | 0 | -308 | -482 | -421 | -503 | -430 | -537 | -473 | -372 | -430 | -555 | -467 | -558 | -555 | -583 |
| -2 | 6 | 0 | -439 | -546 | -424 | -558 | -500 | -570 | -564 | -540 | -595 | -518 | -625 | -561 | -543 | -628 |
| -2 | 7 | 0 | -387 | -244 | -397 | -424 | -421 | -439 | -458 | -449 | -467 | -467 | -445 | -381 | -421 | -485 |
| -3 | 1 | 0 | -528 | -552 | -561 | -577 | -485 | -601 | -497 | -543 | -543 | -485 | -616 | -616 | -607 | -647 |
| -3 | 2 | 0 | -458 | -501 | -522 | -519 | -421 | -540 | -537 | -424 | -528 | -586 | -485 | -543 | -446 | -577 |
| -3 | 3 | 0 | -278 | -415 | -440 | -424 | -446 | -351 | -464 | -467 | -470 | -342 | -495 | -360 | -437 | -513 |
| -3 | 4 | 0 | -455 | -290 | -476 | -482 | -476 | -455 | -473 | -506 | -357 | -510 | -433 | -500 | -467 | -345 |
| -3 | 5 | 0 | -482 | -497 | -342 | -442 | -430 | -525 | -479 | -378 | -406 | -570 | -415 | -573 | -561 | -595 |
| -3 | 6 | 0 | -495 | -547 | -525 | -580 | -492 | -571 | -556 | -577 | -608 | -562 | -641 | -586 | -553 | -626 |
| -3 | 7 | 0 | -369 | -421 | -360 | -418 | -430 | -449 | -458 | -461 | -467 | -476 | -434 | -485 | -430 | -461 |
| -4 | 1 | 0 | -583 | -604 | -610 | -583 | -555 | -641 | -543 | -574 | -595 | -506 | -668 | -677 | -662 | -699 |
| -4 | 2 | 0 | -500 | -509 | -525 | -521 | -460 | -515 | -534 | -390 | -518 | -546 | -509 | -497 | -427 | -567 |
| -4 | 3 | 0 | -260 | -425 | -437 | -367 | -428 | -367 | -446 | -452 | -455 | -318 | -467 | -336 | -403 | -495 |
| -4 | 4 | 0 | -528 | -437 | -562 | -574 | -540 | -540 | -565 | -601 | -446 | -601 | -534 | -610 | -610 | -507 |
| -4 | 5 | 0 | -479 | -497 | -412 | -366 | -427 | -528 | -467 | -445 | -403 | -564 | -433 | -580 | -573 | -598 |
| -4 | 6 | 0 | -528 | -592 | -531 | -568 | -507 | -565 | -522 | -583 | -611 | -589 | -632 | -559 | -553 | -614 |
| -4 | 7 | 0 | -336 | -421 | -290 | -327 | -424 | -449 | -452 | -461 | -476 | -479 | -409 | -476 | -479 | -369 |
| -5 | 1 | 0 | -498 | -495 | -525 | -391 | -470 | -559 | -467 | -449 | -476 | -556 | -553 | -583 | -559 | -611 |
| -5 | 2 | 0 | -556 | -449 | -592 | -589 | -522 | -608 | -608 | -501 | -602 | -617 | -623 | -595 | -544 | -653 |
| -5 | 3 | 0 | -418 | -403 | -412 | -272 | -409 | -373 | -440 | -446 | -452 | -357 | -446 | -354 | -412 | -473 |
| -5 | 4 | 0 | -449 | -540 | -546 | -476 | -564 | -568 | -604 | -619 | -500 | -638 | -577 | -644 | -629 | -555 |
| -5 | 5 | 0 | -506 | -473 | -555 | -564 | -457 | -549 | -509 | -586 | -473 | -595 | -613 | -622 | -610 | -638 |
| -5 | 6 | 0 | -485 | -351 | -488 | -375 | -412 | -525 | -555 | -546 | -570 | -540 | -589 | -525 | -500 | -573 |
| -5 | 7 | 0 | -269 | -427 | -330 | -357 | -439 | -464 | -464 | -473 | -491 | -494 | -366 | -500 | -485 | -369 |
| neg | 1 | 0 | -415 | -607 | -460 | -537 | -579 | -595 | -622 | -671 | -531 | -677 | -653 | -683 | -671 | -573 |
| neg | 2 | 0 | -476 | -547 | -440 | -550 | -522 | -544 | -571 | -592 | -516 | -611 | -595 | -623 | -611 | -504 |
| neg | 3 | 0 | -513 | -556 | -488 | -583 | -498 | -507 | -577 | -611 | -549 | -626 | -614 | -635 | -629 | -519 |
| neg | 4 | 0 | -525 | -537 | -531 | -586 | -464 | -501 | -583 | -598 | -559 | -571 | -614 | -632 | -629 | -540 |
| neg | 5 | 0 | -516 | -397 | -531 | -561 | -424 | -442 | -555 | -601 | -543 | -500 | -607 | -622 | -619 | -552 |
| neg | 6 | 0 | -537 | -455 | -564 | -445 | -439 | -455 | -561 | -613 | -583 | -473 | -641 | -641 | -638 | -662 |

**Appendix 5.8 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with filtered sewage sample over 18 h.**

EHC-GAL

| Dilution | Replicate | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 | 14 | 14.5 | 15 | 15.5 | 16 | 16.5 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 16596 | 23939 | 31566 | 38661 | 43880 | 47664 | 50664 | 51921 | 53353 | 53545 | 53826 | 53572 | 53935 | 53826 | 53880 |
| neat | 2 | 14899 | 21452 | 28809 | 35829 | 40770 | 44716 | 47502 | 49492 | 50001 | 50539 | 51369 | 51213 | 51488 | 51634 | 51500 |
| neat | 3 | 22217 | 28483 | 34324 | 39066 | 43598 | 47111 | 48896 | 50669 | 51438 | 51878 | 52470 | 52827 | 52446 | 52937 | 53184 |
| neat | 4 | 15491 | 22733 | 29895 | 37000 | 42283 | 45948 | 48658 | 50532 | 51234 | 51334 | 51606 | 52088 | 52320 | 51856 | 52503 |
| neat | 5 | 2561 | 5042 | 8826 | 13523 | 20646 | 28120 | 35246 | 40934 | 45280 | 48042 | 50331 | 51719 | 52421 | 53770 | 53795 |
| neat | 6 | 4719 | 8878 | 14344 | 20197 | 27479 | 34187 | 39921 | 43730 | 46358 | 48787 | 49840 | 50359 | 51500 | 51518 | 52132 |
| neat | 7 | 9430 | 14350 | 20377 | 26899 | 32948 | 38865 | 43553 | 47685 | 49611 | 51533 | 52440 | 52785 | 52507 | 52946 | 53074 |
| -1 | 1 | -498 | -659 | -653 | -626 | -656 | -595 | -559 | -513 | -327 | -287 | -61 | 378 | 760 | 1294 | 1892 |
| -1 | 2 | -375 | -528 | -531 | -399 | -531 | -525 | -534 | -357 | -518 | -512 | -369 | -436 | -259 | -332 | -256 |
| -1 | 3 | 449 | 1468 | 3397 | 6266 | 10584 | 15900 | 21797 | 28059 | 34178 | 42088 | 47658 | 51201 | 52818 | 53917 | 54103 |
| -1 | 4 | 3906 | 6687 | 10547 | 15393 | 20557 | 26102 | 32127 | 37898 | 43635 | 46644 | 48664 | 49952 | 50621 | 51036 | 51930 |
| -1 | 5 | -354 | -199 | 64 | 439 | 1166 | 1974 | 3284 | 5313 | 8536 | 12857 | 19882 | 31724 | 42149 | 48399 | 51396 |
| -1 | 6 | -586 | -528 | -363 | -85 | 806 | 2558 | 6650 | 13044 | 21601 | 31800 | 40504 | 46498 | 50185 | 51149 | 52065 |
| -1 | 7 | -336 | -428 | -342 | -428 | -428 | -388 | -183 | -46 | 247 | 799 | 1578 | 2731 | 3815 | 5273 | 6863 |
| -2 | 1 | -540 | -668 | -671 | -659 | -677 | -537 | -668 | -549 | -577 | -628 | -705 | -677 | -696 | -696 | -570 |
| -2 | 2 | -393 | -540 | -555 | -418 | -558 | -570 | -580 | -436 | -589 | -586 | -424 | -506 | -595 | -613 | -601 |
| -2 | 3 | -489 | -538 | -516 | -525 | -519 | -519 | -443 | -376 | -507 | -406 | -504 | -501 | -412 | -504 | -528 |
| -2 | 4 | 733 | 1902 | 3617 | 6028 | 8842 | 12214 | 16126 | 21101 | 30934 | 38988 | 44337 | 47487 | 48930 | 50679 | 51729 |
| -2 | 5 | -546 | -567 | -598 | -616 | -549 | -610 | -604 | -564 | -467 | -628 | -619 | -613 | -567 | -628 | -476 |
| -2 | 6 | -641 | -604 | -543 | -677 | -641 | -650 | -680 | -671 | -677 | -671 | -659 | -662 | -570 | -702 | -689 |
| -2 | 7 | -415 | -500 | -491 | -503 | -415 | -375 | -406 | -513 | -522 | -516 | -555 | -491 | -421 | -568 | -564 |
| -3 | 1 | -528 | -662 | -671 | -671 | -687 | -540 | -699 | -540 | -601 | -561 | -714 | -711 | -717 | -735 | -671 |
| -3 | 2 | -470 | -611 | -617 | -485 | -629 | -647 | -641 | -513 | -653 | -653 | -659 | -522 | -678 | -675 | -681 |
| -3 | 3 | -409 | -418 | -540 | -434 | -553 | -546 | -534 | -415 | -556 | -501 | -479 | -571 | -540 | -580 | -571 |
| -3 | 4 | -305 | -36 | 195 | 553 | 1019 | 1886 | 3205 | 4740 | 6632 | 9116 | 12217 | 15738 | 19468 | 24091 | 28556 |
| -3 | 5 | -543 | -595 | -619 | -628 | -592 | -635 | -641 | -558 | -503 | -653 | -644 | -662 | -638 | -668 | -537 |
| -3 | 6 | -650 | -608 | -516 | -663 | -647 | -638 | -687 | -675 | -699 | -705 | -663 | -647 | -711 | -711 | -730 |
| -3 | 7 | -397 | -510 | -519 | -519 | -418 | -391 | -476 | -510 | -553 | -534 | -568 | -519 | -473 | -424 | -574 |
| -4 | 1 | -592 | -628 | -638 | -714 | -674 | -641 | -744 | -601 | -641 | -662 | -668 | -766 | -772 | -778 | -763 |
| -4 | 2 | -537 | -586 | -582 | -476 | -613 | -619 | -598 | -540 | -634 | -616 | -637 | -561 | -653 | -622 | -628 |
| -4 | 3 | -348 | -348 | -513 | -382 | -528 | -531 | -531 | -409 | -550 | -522 | -425 | -559 | -556 | -428 | -425 |
| -4 | 4 | -586 | -488 | -662 | -659 | -671 | -675 | -681 | -580 | -665 | -675 | -549 | -562 | -711 | -556 | -717 |
| -4 | 5 | -564 | -604 | -616 | -625 | -592 | -634 | -638 | -570 | -561 | -647 | -656 | -671 | -665 | -671 | -616 |
| -4 | 6 | -650 | -565 | -632 | -675 | -614 | -571 | -647 | -629 | -495 | -403 | 64 | 604 | 1184 | 2282 | 3805 |
| -4 | 7 | -427 | -528 | -531 | -540 | -394 | -430 | -522 | -534 | -556 | -437 | -534 | -543 | -543 | -528 | -458 |
| -5 | 1 | -516 | -458 | -489 | -626 | -486 | -577 | -479 | -501 | -528 | -580 | -483 | -638 | -653 | -650 | -660 |
| -5 | 2 | -583 | -657 | -653 | -528 | -666 | -675 | -620 | -547 | -687 | -635 | -693 | -690 | -693 | -605 | -657 |
| -5 | 3 | -336 | -382 | -482 | -382 | -495 | -498 | -485 | -388 | -501 | -492 | -464 | -476 | -522 | -464 | -473 |
| -5 | 4 | -629 | -561 | -674 | -677 | -696 | -677 | -696 | -647 | -696 | -696 | -592 | -586 | -714 | -735 | -745 |
| -5 | 5 | -619 | -653 | -668 | -677 | -659 | -680 | -665 | -631 | -689 | -625 | -714 | -708 | -717 | -604 | -689 |
| -5 | 6 | -610 | -470 | -638 | -638 | -519 | -485 | -546 | -628 | -628 | -656 | -583 | -662 | -662 | -534 | -662 |
| -5 | 7 | -409 | -528 | -531 | -528 | -388 | -439 | -543 | -531 | -562 | -397 | -412 | -555 | -568 | -568 | -500 |
| neg | 1 | -659 | -738 | -723 | -726 | -729 | -704 | -735 | -741 | -738 | -714 | -753 | -601 | -723 | -775 | -762 |
| neg | 2 | -611 | -659 | -653 | -656 | -678 | -626 | -681 | -690 | -687 | -653 | -708 | -629 | -592 | -724 | -663 |
| neg | 3 | -611 | -678 | -641 | -681 | -681 | -629 | -672 | -699 | -702 | -583 | -717 | -751 | -635 | -711 | -611 |
| neg | 4 | -601 | -665 | -571 | -669 | -672 | -571 | -623 | -693 | -702 | -562 | -696 | -727 | -720 | -669 | -662 |
| neg | 5 | -537 | -647 | -571 | -647 | -644 | -525 | -531 | -674 | -680 | -619 | -616 | -708 | -726 | -571 | -732 |
| neg | 6 | -537 | -656 | -705 | -674 | -619 | -626 | -705 | -699 | -693 | -708 | -629 | -723 | -726 | -735 | -741 |

**Appendix 5.8 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with filtered sewage sample over 18 h.**

EHC-GAL

| Dilution | Replicate | 17.5 | 18 |
|---|---|---|---|
| neat | 1 | 53865 | 54613 |
| neat | 2 | 51405 | 51436 |
| neat | 3 | 52629 | 53123 |
| neat | 4 | 52424 | 52549 |
| neat | 5 | 54378 | 54741 |
| neat | 6 | 51753 | 51955 |
| neat | 7 | 52955 | 53419 |
| -1 | 1 | 2972 | 4367 |
| -1 | 2 | -119 | 61 |
| -1 | 3 | 53633 | 54015 |
| -1 | 4 | 51390 | 52195 |
| -1 | 5 | 53056 | 54026 |
| -1 | 6 | 52571 | 52870 |
| -1 | 7 | 8795 | 10757 |
| -2 | 1 | -702 | -729 |
| -2 | 2 | -537 | -616 |
| -2 | 3 | -397 | -550 |
| -2 | 4 | 51659 | 51994 |
| -2 | 5 | -644 | -653 |
| -2 | 6 | -564 | -607 |
| -2 | 7 | -543 | -586 |
| -3 | 1 | -744 | -760 |
| -3 | 2 | -543 | -696 |
| -3 | 3 | -556 | -586 |
| -3 | 4 | 33720 | 38706 |
| -3 | 5 | -564 | -693 |
| -3 | 6 | -742 | -717 |
| -3 | 7 | -592 | -604 |
| -4 | 1 | -775 | -766 |
| -4 | 2 | -698 | -662 |
| -4 | 3 | -580 | -440 |
| -4 | 4 | -702 | -717 |
| -4 | 5 | -586 | -696 |
| -4 | 6 | 5972 | 8945 |
| -4 | 7 | -617 | -501 |
| -5 | 1 | -550 | -513 |
| -5 | 2 | -721 | -702 |
| -5 | 3 | -534 | -437 |
| -5 | 4 | -677 | -754 |
| -5 | 5 | -696 | -735 |
| -5 | 6 | -683 | -693 |
| -5 | 7 | -592 | -449 |
| neg | 1 | -653 | -775 |
| neg | 2 | -742 | -705 |
| neg | 3 | -745 | -635 |
| neg | 4 | -723 | -656 |
| neg | 5 | -647 | -723 |
| neg | 6 | -680 | -751 |

**Appendix 5.8 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with filtered sewage sample over 18 h.**

4-MU-GAL

| Dilution | Replicate | 0 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 0 | 16358 | 16285 | 16383 | 16325 | 16395 | 16383 | 16416 | 16538 | 16441 | 16511 | 16627 | 16941 | 17237 | 16990 |
| neat | 2 | 0 | 77 | 95 | 113 | 147 | 196 | 257 | 312 | 400 | 540 | 888 | 1786 | 3742 | 7157 | 11756 |
| neat | 3 | 0 | -73 | -21 | -30 | 86 | 67 | 263 | 244 | 244 | 357 | 574 | 1032 | 1819 | 3571 | 6925 |
| neat | 4 | 0 | -39 | -30 | 19 | 61 | 177 | 131 | 244 | 321 | 339 | 455 | 980 | 1639 | 3250 | 5591 |
| neat | 5 | 0 | -183 | -195 | -177 | -183 | -67 | -150 | -140 | -113 | -58 | 58 | 296 | 793 | 1874 | 3445 |
| neat | 6 | 0 | -168 | -80 | 9 | -10 | -107 | 70 | -25 | 15 | 0 | 85 | 271 | 756 | 1904 | 4019 |
| neat | 7 | 0 | -125 | 6 | -42 | -61 | -36 | 49 | 55 | 40 | 92 | 190 | 437 | 1087 | 2460 | 4633 |
| -1 | 1 | 0 | -250 | -296 | -265 | -262 | -268 | -262 | -229 | -134 | 141 | 440 | 1496 | 3882 | 8295 | 15452 |
| -1 | 2 | 0 | -186 | -278 | -278 | -281 | -269 | -260 | -260 | -101 | -247 | -89 | -73 | -220 | -73 | -95 |
| -1 | 3 | 0 | -372 | -262 | -369 | -192 | -366 | -198 | -289 | -341 | -329 | -314 | -274 | -299 | -265 | -210 |
| -1 | 4 | 0 | -226 | -113 | -229 | -208 | -144 | -119 | -119 | -55 | -132 | -52 | -40 | -83 | 125 | 223 |
| -1 | 5 | 0 | -296 | -155 | -287 | -198 | -225 | -283 | -293 | -280 | -265 | -287 | -277 | -259 | -134 | -168 |
| -1 | 6 | 0 | -232 | -196 | -159 | -187 | -193 | -37 | -126 | -86 | -144 | -113 | -104 | -86 | -46 | 3 |
| -1 | 7 | 0 | -268 | -262 | -265 | -116 | -280 | -241 | -241 | -265 | -265 | -265 | -262 | -146 | -189 | -216 |
| -2 | 1 | 0 | -144 | -162 | -135 | -116 | -98 | -67 | 88 | -22 | 24 | 52 | 277 | 552 | 1916 | 4962 |
| -2 | 2 | 0 | -315 | -327 | -333 | -361 | -327 | -330 | -333 | -214 | -330 | -211 | -177 | -324 | -220 | -254 |
| -2 | 3 | 0 | -342 | -278 | -348 | -339 | -354 | -214 | -305 | -339 | -345 | -333 | -336 | -312 | -327 | -342 |
| -2 | 4 | 0 | -269 | -272 | -299 | -272 | -232 | -126 | -232 | -199 | -241 | -119 | -214 | -251 | -132 | -254 |
| -2 | 5 | 0 | -314 | -290 | -318 | -211 | -284 | -314 | -302 | -308 | -296 | -299 | -302 | -287 | -183 | -256 |
| -2 | 6 | 0 | -287 | -306 | -312 | -306 | -315 | -159 | -269 | -156 | -315 | -293 | -309 | -318 | -324 | -318 |
| -2 | 7 | 0 | -125 | -302 | -299 | -256 | -302 | -275 | -275 | -293 | -299 | -296 | -275 | -205 | -241 | -287 |
| -3 | 1 | 0 | -272 | -327 | -320 | -327 | -327 | -244 | -195 | -314 | -323 | -314 | -217 | -275 | -195 | -168 |
| -3 | 2 | 0 | -317 | -152 | -256 | -177 | -259 | -308 | -320 | -250 | -296 | -247 | -253 | -302 | -158 | -250 |
| -3 | 3 | 0 | -306 | -351 | -345 | -336 | -342 | -214 | -303 | -336 | -342 | -336 | -336 | -342 | -330 | -348 |
| -3 | 4 | 0 | -125 | -283 | -277 | -122 | -259 | -152 | -219 | -247 | -271 | -161 | -265 | -265 | -112 | -271 |
| -3 | 5 | 0 | -315 | -330 | -315 | -306 | -315 | -333 | -321 | -315 | -309 | -321 | -327 | -315 | -205 | -293 |
| -3 | 6 | 0 | -198 | -308 | -320 | -296 | -299 | -210 | -265 | -207 | -314 | -238 | -290 | -305 | -302 | -314 |
| -3 | 7 | 0 | -226 | -317 | -308 | -302 | -317 | -290 | -274 | -281 | -305 | -311 | -189 | -165 | -274 | -302 |
| -4 | 1 | 0 | -253 | -342 | -336 | -339 | -308 | -201 | -299 | -339 | -336 | -293 | -287 | -327 | -305 | -235 |
| -4 | 2 | 0 | -293 | -250 | -149 | -271 | -250 | -311 | -308 | -287 | -299 | -275 | -253 | -314 | -159 | -250 |
| -4 | 3 | 0 | -214 | -354 | -311 | -330 | -345 | -192 | -311 | -333 | -336 | -333 | -330 | -327 | -318 | -333 |
| -4 | 4 | 0 | -245 | -290 | -266 | -266 | -275 | -168 | -226 | -281 | -278 | -223 | -284 | -275 | -150 | -281 |
| -4 | 5 | 0 | -171 | -308 | -321 | -315 | -321 | -308 | -324 | -193 | -308 | -318 | -327 | -318 | -174 | -284 |
| -4 | 6 | 0 | -183 | -302 | -330 | -253 | -317 | -299 | -275 | -317 | -336 | -205 | -293 | -330 | -321 | -330 |
| -4 | 7 | 0 | -290 | -232 | -308 | -314 | -308 | -305 | -272 | -195 | -320 | -317 | -168 | -174 | -247 | -317 |
| -5 | 1 | 0 | -177 | -324 | -311 | -330 | -253 | -220 | -314 | -327 | -321 | -263 | -263 | -314 | -305 | -147 |
| -5 | 2 | 0 | -164 | -317 | -271 | -326 | -171 | -323 | -320 | -326 | -320 | -302 | -296 | -317 | -207 | -274 |
| -5 | 3 | 0 | -254 | -309 | -248 | -296 | -333 | -156 | -287 | -306 | -324 | -315 | -324 | -315 | -299 | -306 |
| -5 | 4 | 0 | -280 | -167 | -174 | -296 | -286 | -222 | -253 | -293 | -286 | -259 | -293 | -286 | -189 | -271 |
| -5 | 5 | 0 | -281 | -177 | -199 | -351 | -342 | -342 | -342 | -193 | -336 | -339 | -348 | -348 | -190 | -306 |
| -5 | 6 | 0 | -317 | -177 | -317 | -180 | -305 | -333 | -281 | -333 | -330 | -192 | -253 | -327 | -323 | -320 |
| -5 | 7 | 0 | -309 | -165 | -315 | -309 | -309 | -306 | -281 | -171 | -321 | -312 | -174 | -202 | -263 | -306 |
| neg | 1 | 0 | -253 | -302 | -272 | -317 | -168 | -275 | -320 | -320 | -299 | -229 | -253 | -323 | -311 | -195 |
| neg | 2 | 0 | -317 | -250 | -219 | -299 | -183 | -286 | -341 | -332 | -320 | -268 | -256 | -332 | -326 | -265 |
| neg | 3 | 0 | -299 | -140 | -137 | -183 | -155 | -265 | -302 | -308 | -180 | -283 | -204 | -296 | -308 | -235 |
| neg | 4 | 0 | -314 | -333 | -314 | -330 | -174 | -272 | -330 | -330 | -320 | -327 | -177 | -330 | -327 | -259 |
| neg | 5 | 0 | -265 | -338 | -341 | -345 | -235 | -253 | -345 | -332 | -320 | -323 | -171 | -329 | -329 | -277 |
| neg | 6 | 0 | -174 | -321 | -342 | -333 | -324 | -208 | -342 | -318 | -330 | -330 | -330 | -339 | -333 | -232 |

**Appendix 5.8 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with filtered sewage sample over 18 h.**

**4-MU-GAL**

| Dilution | Replicate | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 | 14 | 14.5 | 15 | 15.5 | 16 | 16.5 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 17457 | 21403 | 25862 | 30058 | 32530 | 33397 | 33894 | 34022 | 33986 | 34013 | 33949 | 33912 | 33909 | 33900 | 33894 |
| neat | 2 | 17237 | 22816 | 28037 | 32743 | 35997 | 39048 | 40306 | 41386 | 41963 | 42357 | 42558 | 43165 | 43315 | 43785 | 44255 |
| neat | 3 | 11972 | 17893 | 23984 | 29212 | 33674 | 37055 | 39357 | 40605 | 41432 | 42115 | 42585 | 43620 | 43599 | 44218 | 44911 |
| neat | 4 | 9055 | 13154 | 17924 | 22941 | 28263 | 32670 | 36140 | 38832 | 39994 | 40748 | 41209 | 41954 | 41948 | 42994 | 42979 |
| neat | 5 | 6046 | 9397 | 13709 | 18982 | 24396 | 30262 | 35239 | 39344 | 41389 | 42478 | 43498 | 43717 | 44493 | 44395 | 45228 |
| neat | 6 | 7370 | 11737 | 16742 | 21927 | 27097 | 31836 | 35566 | 38059 | 40186 | 41486 | 42380 | 43149 | 43165 | 43607 | 44413 |
| neat | 7 | 8149 | 12650 | 18302 | 23799 | 29536 | 33918 | 37105 | 39268 | 40770 | 41438 | 41960 | 42076 | 42866 | 43056 | 43556 |
| -1 | 1 | 24280 | 31690 | 36604 | 39320 | 40119 | 40879 | 41337 | 41420 | 42201 | 42601 | 43010 | 43534 | 43791 | 44285 | 45219 |
| -1 | 2 | 9 | -46 | 128 | 580 | 973 | 1929 | 3213 | 5279 | 8221 | 11908 | 16040 | 20450 | 24341 | 28299 | 31949 |
| -1 | 3 | -12 | 730 | 2970 | 7661 | 15290 | 24027 | 31431 | 36656 | 39738 | 41078 | 41804 | 42598 | 42937 | 42854 | 43535 |
| -1 | 4 | 616 | 1147 | 2133 | 4205 | 7248 | 10852 | 16138 | 23166 | 29926 | 36179 | 40687 | 42927 | 43385 | 44126 | 44193 |
| -1 | 5 | 10 | 129 | 522 | 1212 | 2005 | 3342 | 5417 | 8762 | 12922 | 17283 | 21674 | 25471 | 29304 | 32167 | 35206 |
| -1 | 6 | 109 | 308 | 607 | 1159 | 2163 | 3256 | 4831 | 6799 | 9183 | 11941 | 15140 | 18900 | 22571 | 26270 | 30039 |
| -1 | 7 | -149 | -94 | 184 | 516 | 1471 | 3476 | 8875 | 16837 | 24406 | 30000 | 33519 | 35478 | 37144 | 37877 | 38478 |
| -2 | 1 | 11029 | 19223 | 27994 | 33979 | 37589 | 39191 | 39735 | 40363 | 40467 | 40967 | 41090 | 41840 | 41788 | 42545 | 42783 |
| -2 | 2 | -168 | -318 | -318 | -159 | -303 | -174 | -287 | -293 | -257 | -272 | -296 | -263 | -272 | -251 | -260 |
| -2 | 3 | -339 | -333 | -330 | -324 | -305 | -159 | -257 | -217 | -156 | 55 | 70 | 262 | 635 | 1187 | 1919 |
| -2 | 4 | -220 | -251 | -235 | -241 | -64 | -199 | -193 | -52 | -132 | -95 | -34 | 58 | 109 | 286 | 323 |
| -2 | 5 | -177 | -232 | -195 | -70 | -116 | -34 | 73 | 210 | 354 | 561 | 882 | 1132 | 1550 | 1938 | 2475 |
| -2 | 6 | -312 | -309 | -260 | -321 | -174 | -306 | -303 | -174 | -287 | -290 | -287 | -269 | -211 | -119 | -248 |
| -2 | 7 | -244 | -281 | -128 | -198 | -49 | 174 | 647 | 1282 | 2182 | 3513 | 4990 | 6683 | 8811 | 11011 | 13153 |
| -3 | 1 | 171 | 1581 | 5744 | 13504 | 22352 | 29884 | 35291 | 38819 | 39555 | 40223 | 40586 | 41068 | 41603 | 41651 | 41984 |
| -3 | 2 | -183 | -299 | -293 | -165 | -192 | -140 | -259 | -274 | -268 | -259 | -277 | -207 | -265 | -235 | -244 |
| -3 | 3 | -348 | -342 | -333 | -342 | -293 | -196 | -214 | -321 | -324 | -174 | -199 | -327 | -306 | -226 | -318 |
| -3 | 4 | -250 | -274 | -259 | -265 | -125 | -250 | -259 | -134 | -125 | -247 | -247 | -158 | -244 | -241 | -231 |
| -3 | 5 | -226 | -300 | -300 | -214 | -318 | -309 | -293 | -226 | -165 | -10 | 277 | 491 | 976 | 1452 | 2111 |
| -3 | 6 | -311 | -290 | -213 | -311 | -155 | -302 | -308 | -238 | -256 | -296 | -296 | -296 | -146 | -189 | -287 |
| -3 | 7 | -265 | -311 | -146 | -256 | -232 | -219 | -140 | -232 | -256 | -174 | -201 | -250 | -281 | -238 | -293 |
| -4 | 1 | -333 | -323 | -333 | -220 | -299 | -159 | -162 | -296 | -296 | -296 | -241 | -305 | -290 | -278 | -265 |
| -4 | 2 | -180 | -299 | -302 | -162 | -259 | -140 | -183 | -296 | -290 | -287 | -296 | -171 | -284 | -213 | -241 |
| -4 | 3 | -321 | -324 | -327 | -321 | -217 | -238 | -202 | -314 | -305 | -229 | -205 | -321 | -305 | -284 | -296 |
| -4 | 4 | -260 | -281 | -248 | -284 | -132 | -254 | -278 | -199 | -135 | -263 | -266 | -110 | -254 | -266 | -220 |
| -4 | 5 | -217 | -217 | -257 | -180 | -315 | -312 | -293 | -269 | -293 | -302 | -135 | -278 | -147 | -281 | -296 |
| -4 | 6 | -336 | -281 | -183 | -333 | -180 | -333 | -324 | -263 | -263 | -330 | -333 | -317 | -217 | -308 | -314 |
| -4 | 7 | -259 | -305 | -293 | -287 | -253 | -207 | -287 | -226 | -268 | -281 | -140 | -153 | -296 | -302 | -305 |
| -5 | 1 | -296 | -281 | -263 | -198 | -208 | -101 | -18 | 58 | 223 | 485 | 900 | 1501 | 2515 | 4404 | 7565 |
| -5 | 2 | -210 | -320 | -305 | -186 | -253 | -171 | -143 | -274 | -283 | -283 | -280 | -110 | -268 | -119 | -198 |
| -5 | 3 | -309 | -309 | -296 | -296 | -184 | -235 | -217 | -260 | -254 | -226 | -223 | -132 | -263 | -260 | -165 |
| -5 | 4 | -256 | -271 | -238 | -265 | -97 | -238 | -244 | -174 | -116 | -216 | -225 | -201 | -216 | -222 | -155 |
| -5 | 5 | -193 | -208 | -208 | -187 | -342 | -336 | -299 | -284 | -336 | -315 | -232 | -263 | -244 | -260 | -327 |
| -5 | 6 | -327 | -311 | -168 | -327 | -192 | -327 | -296 | -250 | -223 | -314 | -323 | -327 | -235 | -314 | -320 |
| -5 | 7 | -232 | -287 | -312 | -275 | -306 | -153 | -312 | -156 | -251 | -296 | -257 | -303 | -303 | -303 | -303 |
| neg | 1 | -290 | -308 | -229 | -290 | -296 | -278 | -284 | -284 | -268 | -265 | -275 | -168 | -256 | -247 | -201 |
| neg | 2 | -171 | -323 | -201 | -329 | -326 | -317 | -323 | -314 | -293 | -302 | -308 | -152 | -174 | -296 | -277 |
| neg | 3 | -161 | -296 | -253 | -305 | -293 | -280 | -286 | -216 | -189 | -231 | -286 | -204 | -192 | -286 | -283 |
| neg | 4 | -256 | -323 | -314 | -330 | -317 | -317 | -317 | -259 | -314 | -189 | -253 | -272 | -265 | -317 | -308 |
| neg | 5 | -271 | -329 | -326 | -335 | -317 | -329 | -168 | -174 | -320 | -183 | -161 | -293 | -302 | -174 | -323 |
| neg | 6 | -290 | -327 | -324 | -333 | -281 | -293 | -196 | -165 | -318 | -205 | -190 | -306 | -318 | -202 | -309 |

**Appendix 5.8 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with filtered sewage sample over 18 h.**

**4-MU-GAL**

| Dilution | Replicate | 17.5 | 18 |
|---|---|---|---|
| neat | 1 | 33949 | 33986 |
| neat | 2 | 44789 | 45131 |
| neat | 3 | 44850 | 45475 |
| neat | 4 | 43782 | 44157 |
| neat | 5 | 45088 | 45509 |
| neat | 6 | 44675 | 44504 |
| neat | 7 | 43861 | 43837 |
| -1 | 1 | 45765 | 46092 |
| -1 | 2 | 34742 | 37009 |
| -1 | 3 | 43471 | 44063 |
| -1 | 4 | 44755 | 45158 |
| -1 | 5 | 37269 | 39183 |
| -1 | 6 | 33106 | 35688 |
| -1 | 7 | 38954 | 39183 |
| -2 | 1 | 43171 | 43464 |
| -2 | 2 | -251 | -153 |
| -2 | 3 | 3247 | 5005 |
| -2 | 4 | 503 | 833 |
| -2 | 5 | 3046 | 3708 |
| -2 | 6 | -275 | -129 |
| -2 | 7 | 15598 | 18039 |
| -3 | 1 | 42118 | 42420 |
| -3 | 2 | -250 | -256 |
| -3 | 3 | -312 | -330 |
| -3 | 4 | -238 | -88 |
| -3 | 5 | 2945 | 3875 |
| -3 | 6 | -293 | -171 |
| -3 | 7 | -296 | -274 |
| -4 | 1 | -211 | -122 |
| -4 | 2 | -241 | -287 |
| -4 | 3 | -311 | -305 |
| -4 | 4 | -269 | -199 |
| -4 | 5 | -159 | -217 |
| -4 | 6 | -247 | -253 |
| -4 | 7 | -281 | -275 |
| -5 | 1 | 12415 | 18973 |
| -5 | 2 | -125 | -274 |
| -5 | 3 | -257 | -257 |
| -5 | 4 | -201 | -216 |
| -5 | 5 | -333 | -196 |
| -5 | 6 | -195 | -287 |
| -5 | 7 | -232 | -202 |
| neg | 1 | -265 | -244 |
| neg | 2 | -131 | -296 |
| neg | 3 | -244 | -280 |
| neg | 4 | -305 | -305 |
| neg | 5 | -320 | -229 |
| neg | 6 | -306 | -159 |

## Appendix 5.9: Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with final sewage sample over 18 h.

EHC-GAL

| Dilution | Replicate | 0 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 0 | -68 | -95 | -241 | -245 | -119 | -187 | -266 | -269 | -245 | -202 | -263 | -193 | -159 | 299 |
| neat | 2 | 0 | -247 | -97 | -253 | -277 | -180 | -256 | -174 | -225 | -167 | -177 | -79 | 107 | 220 | 650 |
| neat | 3 | 0 | -73 | -207 | -214 | -192 | -165 | -104 | -226 | -204 | -201 | -183 | 40 | 113 | 681 | 1517 |
| neat | 4 | 0 | -144 | -186 | -195 | -202 | -92 | -37 | -211 | -128 | -83 | -137 | 43 | 397 | 1297 | 3217 |
| neat | 5 | 0 | -312 | -339 | -367 | -360 | -388 | -318 | -376 | -272 | -367 | -406 | -394 | -388 | -238 | -80 |
| neat | 6 | 0 | -110 | -235 | -272 | -281 | -284 | -265 | -308 | -156 | -327 | -317 | -330 | -357 | -272 | -317 |
| neat | 7 | 0 | -73 | -247 | -100 | -284 | -195 | -305 | -308 | -262 | -326 | -204 | -210 | -342 | -335 | -360 |
| -1 | 1 | 0 | -192 | -235 | -348 | -354 | -314 | -247 | -394 | -418 | -262 | -366 | -433 | -406 | -449 | -418 |
| -1 | 2 | 0 | -385 | -296 | -418 | -440 | -415 | -446 | -339 | -431 | -452 | -467 | -351 | -379 | -486 | -455 |
| -1 | 3 | 0 | -192 | -247 | -260 | -253 | -253 | -247 | -150 | -318 | -202 | -342 | -269 | -272 | -205 | -366 |
| -1 | 4 | 0 | -269 | -272 | -287 | -311 | -174 | -168 | -327 | -259 | -275 | -357 | -272 | -388 | -382 | -293 |
| -1 | 5 | 0 | -333 | -296 | -369 | -385 | -391 | -327 | -418 | -327 | -403 | -458 | -461 | -467 | -324 | -427 |
| -1 | 6 | 0 | -170 | -305 | -347 | -357 | -354 | -344 | -399 | -235 | -430 | -412 | -405 | -442 | -384 | -424 |
| -1 | 7 | 0 | -119 | -250 | -104 | -281 | -146 | -311 | -326 | -296 | -342 | -247 | -299 | -345 | -317 | -284 |
| -2 | 1 | 0 | -186 | -204 | -277 | -268 | -262 | -137 | -192 | -332 | -207 | -320 | -357 | -329 | -405 | -372 |
| -2 | 2 | 0 | -208 | -251 | -245 | -263 | -257 | -263 | -147 | -254 | -318 | -309 | -220 | -333 | -312 | -202 |
| -2 | 3 | 0 | -225 | -106 | -265 | -259 | -274 | -271 | -198 | -308 | -198 | -311 | -335 | -207 | -259 | -369 |
| -2 | 4 | 0 | -284 | -287 | -317 | -332 | -189 | -335 | -335 | -253 | -277 | -363 | -314 | -400 | -409 | -287 |
| -2 | 5 | 0 | -253 | -128 | -308 | -308 | -323 | -293 | -357 | -329 | -360 | -384 | -400 | -409 | -302 | -393 |
| -2 | 6 | 0 | -257 | -315 | -415 | -421 | -421 | -397 | -461 | -464 | -498 | -473 | -412 | -516 | -452 | -443 |
| -2 | 7 | 0 | -158 | -253 | -128 | -283 | -198 | -311 | -311 | -332 | -347 | -262 | -347 | -375 | -366 | -274 |
| -3 | 1 | 0 | -183 | -226 | -259 | -259 | -262 | -134 | -146 | -317 | -207 | -317 | -250 | -336 | -363 | -372 |
| -3 | 2 | 0 | -205 | -241 | -244 | -275 | -260 | -266 | -192 | -241 | -315 | -308 | -336 | -315 | -296 | -235 |
| -3 | 3 | 0 | -326 | -174 | -363 | -363 | -375 | -357 | -360 | -338 | -354 | -320 | -439 | -341 | -366 | -305 |
| -3 | 4 | 0 | -348 | -351 | -394 | -406 | -281 | -443 | -394 | -308 | -391 | -446 | -482 | -479 | -488 | -461 |
| -3 | 5 | 0 | -122 | -128 | -296 | -305 | -311 | -277 | -341 | -305 | -344 | -372 | -378 | -390 | -296 | -390 |
| -3 | 6 | 0 | -287 | -168 | -336 | -345 | -321 | -303 | -385 | -379 | -425 | -400 | -284 | -452 | -361 | -296 |
| -3 | 7 | 0 | -262 | -311 | -231 | -317 | -280 | -363 | -289 | -387 | -415 | -302 | -430 | -439 | -436 | -320 |
| -4 | 1 | 0 | -198 | -232 | -247 | -259 | -256 | -162 | -195 | -308 | -250 | -299 | -204 | -333 | -348 | -366 |
| -4 | 2 | 0 | -198 | -263 | -269 | -284 | -290 | -284 | -318 | -202 | -354 | -336 | -366 | -376 | -327 | -388 |
| -4 | 3 | 0 | -354 | -269 | -364 | -394 | -391 | -406 | -428 | -290 | -406 | -315 | -473 | -422 | -422 | -440 |
| -4 | 4 | 0 | -324 | -309 | -385 | -403 | -406 | -425 | -354 | -284 | -348 | -422 | -480 | -449 | -480 | -495 |
| -4 | 5 | 0 | -116 | -195 | -302 | -311 | -278 | -284 | -354 | -339 | -360 | -372 | -327 | -403 | -262 | -427 |
| -4 | 6 | 0 | -253 | -275 | -281 | -296 | -263 | -247 | -305 | -330 | -375 | -339 | -379 | -397 | -281 | -406 |
| -4 | 7 | 0 | -385 | -315 | -333 | -449 | -425 | -480 | -342 | -516 | -525 | -449 | -541 | -553 | -550 | -504 |
| -5 | 1 | 0 | -171 | -217 | -229 | -241 | -272 | -162 | -244 | -186 | -250 | -308 | -278 | -351 | -299 | -345 |
| -5 | 2 | 0 | -190 | -266 | -293 | -293 | -302 | -299 | -324 | -202 | -354 | -342 | -364 | -370 | -315 | -391 |
| -5 | 3 | 0 | -284 | -287 | -281 | -345 | -367 | -370 | -394 | -287 | -394 | -275 | -434 | -400 | -416 | -446 |
| -5 | 4 | 0 | -152 | -158 | -259 | -271 | -274 | -308 | -174 | -204 | -207 | -302 | -351 | -308 | -351 | -390 |
| -5 | 5 | 0 | -141 | -226 | -302 | -305 | -186 | -287 | -348 | -333 | -363 | -370 | -238 | -394 | -260 | -425 |
| -5 | 6 | 0 | -232 | -269 | -278 | -299 | -183 | -186 | -293 | -327 | -333 | -321 | -372 | -379 | -238 | -394 |
| -5 | 7 | 0 | -293 | -162 | -269 | -360 | -351 | -397 | -284 | -418 | -449 | -318 | -452 | -455 | -467 | -449 |
| neg | 1 | 0 | -64 | -58 | -238 | -263 | -266 | -299 | -205 | -311 | -153 | -244 | -324 | -263 | -311 | -366 |
| neg | 2 | 0 | -137 | -79 | -235 | -262 | -256 | -284 | -241 | -296 | -158 | -232 | -290 | -250 | -299 | -345 |
| neg | 3 | 0 | -226 | -242 | -248 | -293 | -293 | -318 | -321 | -339 | -190 | -220 | -278 | -251 | -300 | -370 |
| neg | 4 | 0 | -259 | -277 | -274 | -320 | -329 | -354 | -357 | -378 | -253 | -229 | -235 | -241 | -326 | -387 |
| neg | 5 | 0 | -351 | -403 | -379 | -421 | -449 | -467 | -473 | -492 | -409 | -382 | -519 | -412 | -412 | -443 |
| neg | 6 | 0 | -183 | -231 | -216 | -277 | -286 | -308 | -314 | -329 | -347 | -265 | -366 | -357 | -244 | -219 |

## Appendix 5.9 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with final sewage sample over 18 h.

EHC-GAL

| Dilution | Replicate | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 | 14 | 14.5 | 15 | 15.5 | 16 | 16.5 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 1199 | 2871 | 5536 | 9311 | 14065 | 19135 | 24954 | 31424 | 37604 | 45136 | 51197 | 54374 | 55024 | 55711 | 56004 |
| neat | 2 | 1593 | 3046 | 5494 | 9165 | 13163 | 18317 | 24842 | 32948 | 41435 | 48064 | 51146 | 52983 | 53594 | 53908 | 53618 |
| neat | 3 | 3403 | 6687 | 11655 | 17777 | 25724 | 34883 | 43367 | 49162 | 52775 | 53905 | 54637 | 54536 | 54637 | 54164 | 54701 |
| neat | 4 | 6451 | 11676 | 20472 | 32969 | 44904 | 50807 | 53007 | 53752 | 54307 | 54289 | 54576 | 54350 | 54329 | 54579 | 54780 |
| neat | 5 | -80 | 229 | 793 | 1699 | 2911 | 5072 | 8340 | 12332 | 17389 | 23184 | 29187 | 34891 | 40220 | 45124 | 49088 |
| neat | 6 | -293 | -217 | -21 | 467 | 2115 | 5966 | 13333 | 26878 | 44102 | 49955 | 51655 | 52815 | 52559 | 53239 | 52974 |
| neat | 7 | -348 | -290 | -204 | 107 | 791 | 2564 | 7081 | 15125 | 26881 | 41920 | 49248 | 52330 | 52544 | 52834 | 52870 |
| -1 | 1 | -363 | -436 | -476 | -482 | -357 | -473 | -397 | -507 | -345 | -479 | -501 | -488 | -504 | -504 | -516 |
| -1 | 2 | -412 | -522 | -507 | -342 | -446 | -290 | 210 | 1629 | 4574 | 10254 | 19760 | 30893 | 41270 | 48194 | 52506 |
| -1 | 3 | -217 | -388 | -388 | -327 | -272 | -388 | -363 | -327 | -403 | -263 | -348 | -409 | -388 | -257 | -437 |
| -1 | 4 | -363 | -403 | -385 | -372 | -348 | -211 | -165 | 85 | 314 | 641 | 1157 | 1776 | 2652 | 3650 | 4834 |
| -1 | 5 | -498 | -498 | -412 | -430 | -537 | -531 | -525 | -391 | -409 | -528 | -525 | -391 | -577 | -568 | -586 |
| -1 | 6 | -473 | -460 | -463 | -460 | -448 | -320 | 98 | 1203 | 2775 | 4960 | 8643 | 12739 | 16749 | 21220 | 25563 |
| -1 | 7 | -119 | 299 | 1139 | 2790 | 5445 | 9223 | 13956 | 19593 | 25599 | 31233 | 37568 | 43300 | 48076 | 51143 | 51881 |
| -2 | 1 | -351 | -369 | -402 | -409 | -335 | -265 | -412 | -311 | -369 | -445 | -473 | -470 | -326 | -344 | -515 |
| -2 | 2 | -202 | -379 | -385 | -260 | -364 | -367 | -412 | -263 | -278 | -266 | -406 | -412 | -357 | -440 | -327 |
| -2 | 3 | -250 | -131 | -137 | 354 | 849 | 1520 | 2832 | 4746 | 7673 | 11457 | 18546 | 27360 | 35103 | 40687 | 43922 |
| -2 | 4 | -360 | -442 | -439 | -464 | -476 | -357 | -503 | -354 | -409 | -503 | -500 | -531 | -528 | -546 | -558 |
| -2 | 5 | -427 | -445 | -293 | -387 | -479 | -470 | -482 | -451 | -473 | -503 | -512 | -442 | -558 | -384 | -424 |
| -2 | 6 | -543 | -528 | -546 | -559 | -583 | -571 | -589 | -598 | -485 | -595 | -620 | -595 | -641 | -644 | -525 |
| -2 | 7 | -390 | -402 | -418 | -396 | -415 | -433 | -433 | -454 | -317 | -445 | -390 | -473 | -384 | -433 | -503 |
| -3 | 1 | -357 | -372 | -403 | -348 | -369 | -339 | -427 | -342 | -436 | -400 | -467 | -473 | -369 | -391 | -345 |
| -3 | 2 | -199 | -372 | -385 | -351 | -324 | -315 | -412 | -424 | -440 | -281 | -372 | -458 | -354 | -476 | -339 |
| -3 | 3 | -436 | -366 | -497 | -354 | -390 | -509 | -531 | -540 | -476 | -485 | -561 | -558 | -570 | -555 | -589 |
| -3 | 4 | -430 | -528 | -525 | -540 | -540 | -403 | -580 | -580 | -437 | -607 | -580 | -617 | -617 | -635 | -656 |
| -3 | 5 | -412 | -415 | -262 | -384 | -463 | -290 | -473 | -454 | -479 | -488 | -497 | -439 | -525 | -415 | -378 |
| -3 | 6 | -458 | -440 | -470 | -486 | -504 | -473 | -486 | -516 | -391 | -507 | -553 | -498 | -562 | -577 | -483 |
| -3 | 7 | -393 | -466 | -430 | -469 | -485 | -494 | -390 | -378 | -469 | -457 | -512 | -567 | -518 | -546 | -524 |
| -4 | 1 | -369 | -375 | -394 | -244 | -394 | -378 | -430 | -418 | -443 | -308 | -366 | -452 | -439 | -452 | -418 |
| -4 | 2 | -275 | -403 | -418 | -412 | -311 | -281 | -418 | -464 | -458 | -458 | -366 | -491 | -372 | -501 | -525 |
| -4 | 3 | -486 | -440 | -525 | -443 | -489 | -385 | -385 | -516 | -553 | -553 | -599 | -452 | -620 | -614 | -495 |
| -4 | 4 | -358 | -486 | -501 | -528 | -516 | -550 | -547 | -577 | -589 | -562 | -522 | -589 | -574 | -620 | -629 |
| -4 | 5 | -296 | -446 | -293 | -424 | -458 | -375 | -476 | -485 | -504 | -507 | -516 | -482 | -543 | -491 | -510 |
| -4 | 6 | -400 | -385 | -424 | -433 | -458 | -379 | -394 | -455 | -504 | -400 | -491 | -385 | -510 | -513 | -549 |
| -4 | 7 | -403 | -580 | -559 | -583 | -480 | -525 | -596 | -565 | -632 | -504 | -644 | -672 | -669 | -678 | -568 |
| -5 | 1 | -375 | -385 | -421 | -253 | -403 | -406 | -278 | -424 | -430 | -321 | -314 | -409 | -443 | -458 | -449 |
| -5 | 2 | -391 | -391 | -400 | -400 | -263 | -412 | -330 | -428 | -428 | -446 | -293 | -443 | -412 | -440 | -479 |
| -5 | 3 | -467 | -443 | -492 | -434 | -477 | -391 | -443 | -403 | -519 | -513 | -519 | -470 | -553 | -544 | -498 |
| -5 | 4 | -241 | -366 | -387 | -400 | -354 | -433 | -348 | -455 | -464 | -424 | -378 | -433 | -418 | -485 | -461 |
| -5 | 5 | -266 | -434 | -293 | -421 | -415 | -421 | -324 | -489 | -510 | -504 | -522 | -492 | -486 | -522 | -528 |
| -5 | 6 | -357 | -333 | -397 | -400 | -421 | -275 | -433 | -376 | -476 | -314 | -415 | -336 | -470 | -479 | -519 |
| -5 | 7 | -327 | -489 | -385 | -504 | -351 | -391 | -531 | -531 | -547 | -449 | -565 | -470 | -574 | -595 | -565 |
| neg | 1 | -354 | -324 | -348 | -369 | -278 | -409 | -333 | -369 | -437 | -330 | -315 | -339 | -311 | -409 | -336 |
| neg | 2 | -360 | -302 | -342 | -338 | -232 | -387 | -406 | -259 | -421 | -290 | -378 | -284 | -329 | -311 | -418 |
| neg | 3 | -400 | -306 | -379 | -367 | -437 | -409 | -446 | -470 | -412 | -434 | -492 | -486 | -483 | -507 | -525 |
| neg | 4 | -427 | -281 | -390 | -360 | -470 | -400 | -439 | -494 | -384 | -509 | -516 | -537 | -534 | -543 | -552 |
| neg | 5 | -543 | -437 | -476 | -467 | -586 | -452 | -473 | -592 | -604 | -638 | -635 | -653 | -641 | -669 | -669 |
| neg | 6 | -375 | -405 | -283 | -250 | -424 | -421 | -439 | -369 | -473 | -473 | -476 | -494 | -473 | -500 | -442 |

**Appendix 5.9 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with final sewage sample over 18 h.**

EHC-GAL

| Dilution | Replicate | 17.5 | 18 |
|---|---|---|---|
| neat | 1 | 55888 | 55955 |
| neat | 2 | 53282 | 53856 |
| neat | 3 | 54176 | 54518 |
| neat | 4 | 54741 | 54637 |
| neat | 5 | 51994 | 53404 |
| neat | 6 | 52559 | 52882 |
| neat | 7 | 52641 | 52980 |
| -1 | 1 | -537 | -534 |
| -1 | 2 | 53611 | 54661 |
| -1 | 3 | -449 | -397 |
| -1 | 4 | 6384 | 7886 |
| -1 | 5 | -543 | -583 |
| -1 | 6 | 30696 | 37813 |
| -1 | 7 | 52867 | 53133 |
| -2 | 1 | -393 | -518 |
| -2 | 2 | -437 | -318 |
| -2 | 3 | 46947 | 48717 |
| -2 | 4 | -454 | -461 |
| -2 | 5 | -567 | -586 |
| -2 | 6 | -641 | -589 |
| -2 | 7 | -497 | -503 |
| -3 | 1 | -476 | -384 |
| -3 | 2 | -461 | -492 |
| -3 | 3 | -567 | -607 |
| -3 | 4 | -656 | -507 |
| -3 | 5 | -555 | -549 |
| -3 | 6 | -467 | -458 |
| -3 | 7 | -595 | -460 |
| -4 | 1 | -510 | -409 |
| -4 | 2 | -382 | -540 |
| -4 | 3 | -635 | -486 |
| -4 | 4 | -657 | -644 |
| -4 | 5 | -412 | -479 |
| -4 | 6 | -519 | -562 |
| -4 | 7 | -709 | -638 |
| -5 | 1 | -482 | -455 |
| -5 | 2 | -443 | -495 |
| -5 | 3 | -589 | -513 |
| -5 | 4 | -528 | -531 |
| -5 | 5 | -501 | -415 |
| -5 | 6 | -537 | -556 |
| -5 | 7 | -608 | -601 |
| neg | 1 | -434 | -482 |
| neg | 2 | -348 | -439 |
| neg | 3 | -531 | -419 |
| neg | 4 | -577 | -497 |
| neg | 5 | -684 | -690 |
| neg | 6 | -488 | -531 |

**Appendix 5.9 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with final sewage sample over 18 h.**

4-MU-GAL

| Dilution | Replicate | 0 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 0 | 16321 | 16437 | 16403 | 16321 | 16324 | 16355 | 16535 | 16477 | 16434 | 16434 | 16626 | 16614 | 16745 | 17047 |
| neat | 2 | 0 | -287 | -274 | -149 | -284 | -137 | -253 | -274 | -259 | -168 | -137 | -271 | -259 | -131 | -250 |
| neat | 3 | 0 | -281 | -281 | -281 | -275 | -259 | -88 | -232 | -73 | -52 | -67 | 351 | 806 | 1874 | 3974 |
| neat | 4 | 0 | -309 | -306 | -296 | -150 | -293 | -135 | -269 | -150 | -98 | -196 | 85 | 290 | 863 | 1559 |
| neat | 5 | 0 | -89 | -129 | -83 | -214 | -211 | -101 | -58 | -98 | -220 | -58 | -86 | -95 | 64 | 122 |
| neat | 6 | 0 | -229 | -235 | -140 | -113 | -222 | -201 | -85 | -210 | -189 | -164 | -45 | 28 | 385 | 788 |
| neat | 7 | 0 | -34 | -82 | -174 | -70 | -134 | -146 | 27 | -140 | -122 | -116 | -98 | 31 | 31 | -49 |
| -1 | 1 | 0 | -314 | -366 | -375 | -222 | -366 | -250 | -351 | -375 | -354 | -369 | -354 | -329 | -164 | -18 |
| -1 | 2 | 0 | -336 | -323 | -204 | -333 | -198 | -290 | -302 | -271 | -165 | -91 | -125 | 40 | 403 | 623 |
| -1 | 3 | 0 | -247 | -220 | -256 | -143 | -122 | -146 | -259 | -210 | -155 | -265 | -198 | -253 | -143 | -195 |
| -1 | 4 | 0 | -317 | -329 | -335 | -241 | -332 | -174 | -323 | -186 | -195 | -332 | -195 | -290 | -247 | -323 |
| -1 | 5 | 0 | -235 | -235 | -125 | -241 | -247 | -119 | -192 | -144 | -278 | -110 | -244 | -192 | -128 | -235 |
| -1 | 6 | 0 | -213 | -232 | -171 | -131 | -229 | -213 | -67 | -229 | -223 | -217 | -79 | -213 | -149 | -27 |
| -1 | 7 | 0 | -271 | -143 | -305 | -213 | -271 | -299 | -146 | -302 | -296 | -302 | -277 | -161 | -195 | -293 |
| -2 | 1 | 0 | -226 | -244 | -250 | -226 | -143 | -226 | -241 | -250 | -88 | -98 | -235 | -189 | -143 | -229 |
| -2 | 2 | 0 | -229 | -205 | -86 | -253 | -217 | -198 | -220 | -208 | -98 | -113 | -244 | -232 | -116 | -241 |
| -2 | 3 | 0 | -52 | -73 | -152 | -52 | -101 | -128 | -201 | -162 | -119 | -192 | -186 | -195 | -33 | -189 |
| -2 | 4 | 0 | -168 | -186 | -186 | -55 | -192 | -40 | -180 | -86 | -119 | -189 | -171 | -122 | -82 | -168 |
| -2 | 5 | 0 | -302 | -302 | -284 | -311 | -317 | -162 | -345 | -189 | -317 | -171 | -317 | -247 | -171 | -314 |
| -2 | 6 | 0 | -201 | -207 | -174 | -103 | -210 | -210 | -146 | -210 | -210 | -207 | -140 | -210 | -164 | -146 |
| -2 | 7 | 0 | -320 | -268 | -323 | -320 | -272 | -336 | -317 | -336 | -333 | -339 | -272 | -186 | -201 | -339 |
| -3 | 1 | 0 | -196 | -61 | -101 | -217 | -214 | -226 | -211 | -89 | -186 | -138 | -107 | -98 | -205 | -223 |
| -3 | 2 | 0 | -161 | -128 | -67 | -207 | -198 | -149 | -165 | -149 | -49 | -97 | -189 | -189 | -103 | -186 |
| -3 | 3 | 0 | -131 | -119 | -82 | -43 | -137 | -140 | -189 | -183 | -137 | -189 | -195 | -201 | -46 | -186 |
| -3 | 4 | 0 | -147 | -177 | -180 | -55 | -177 | -86 | -147 | -141 | -110 | -180 | -183 | -128 | -98 | -134 |
| -3 | 5 | 0 | -321 | -333 | -321 | -333 | -339 | -196 | -342 | -184 | -336 | -184 | -342 | -242 | -196 | -339 |
| -3 | 6 | 0 | -189 | -189 | -183 | -116 | -219 | -213 | -164 | -216 | -222 | -210 | -183 | -213 | -152 | -177 |
| -3 | 7 | 0 | -210 | -210 | -210 | -189 | -168 | -223 | -226 | -223 | -229 | -223 | -91 | -91 | -116 | -232 |
| -4 | 1 | 0 | -211 | -318 | -309 | -391 | -385 | -379 | -235 | -321 | -382 | -382 | -379 | -361 | -385 | -382 |
| -4 | 2 | 0 | -116 | -73 | -70 | -201 | -204 | -155 | -91 | -128 | -113 | -70 | -143 | -165 | -61 | -119 |
| -4 | 3 | 0 | -147 | -150 | -31 | -31 | -162 | -144 | -180 | -177 | -147 | -177 | -177 | -180 | -34 | -171 |
| -4 | 4 | 0 | -104 | -177 | -196 | -70 | -192 | -192 | -141 | -196 | -202 | -196 | -211 | -86 | -80 | -92 |
| -4 | 5 | 0 | -211 | -220 | -223 | -226 | -223 | -226 | -232 | -70 | -235 | -80 | -229 | -113 | -89 | -229 |
| -4 | 6 | 0 | -177 | -125 | -222 | -155 | -244 | -238 | -216 | -244 | -244 | -238 | -232 | -183 | -174 | -222 |
| -4 | 7 | 0 | -204 | -210 | -186 | -214 | -79 | -220 | -220 | -214 | -226 | -220 | -146 | -113 | -79 | -207 |
| -5 | 1 | 0 | -177 | -241 | -235 | -247 | -256 | -140 | -186 | -241 | -244 | -259 | -250 | -247 | -250 | -92 |
| -5 | 2 | 0 | -67 | -49 | -79 | -208 | -208 | -141 | -76 | -141 | -119 | -98 | -89 | -180 | -70 | -83 |
| -5 | 3 | 0 | -329 | -329 | -216 | -183 | -329 | -326 | -329 | -342 | -320 | -335 | -342 | -339 | -204 | -332 |
| -5 | 4 | 0 | -104 | -168 | -220 | -107 | -220 | -205 | -128 | -214 | -211 | -226 | -226 | -116 | -141 | -80 |
| -5 | 5 | 0 | -207 | -229 | -238 | -241 | -229 | -244 | -247 | -198 | -247 | -143 | -223 | -91 | -146 | -235 |
| -5 | 6 | 0 | -120 | -132 | -269 | -202 | -287 | -297 | -284 | -263 | -300 | -284 | -300 | -257 | -214 | -287 |
| -5 | 7 | 0 | -208 | -226 | -184 | -226 | -83 | -217 | -235 | -193 | -242 | -217 | -235 | -235 | -98 | -138 |
| neg | 1 | 0 | -198 | -211 | -217 | -73 | -147 | -86 | -220 | -226 | -211 | -229 | -217 | -226 | -226 | -153 |
| neg | 2 | 0 | -204 | -214 | -217 | -82 | -61 | -153 | -226 | -223 | -110 | -226 | -79 | -223 | -217 | -201 |
| neg | 3 | 0 | -357 | -372 | -381 | -274 | -265 | -338 | -375 | -381 | -219 | -384 | -341 | -381 | -326 | -378 |
| neg | 4 | 0 | -290 | -241 | -302 | -235 | -247 | -287 | -311 | -311 | -192 | -311 | -296 | -308 | -235 | -302 |
| neg | 5 | 0 | -247 | -98 | -257 | -232 | -232 | -263 | -281 | -272 | -180 | -266 | -272 | -269 | -119 | -272 |
| neg | 6 | 0 | -229 | -141 | -248 | -223 | -232 | -251 | -254 | -235 | -171 | -229 | -263 | -260 | -101 | -251 |

**Appendix 5.9 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with final sewage sample over 18 h.**

**4-MU-GAL**

| Dilution | Replicate | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 | 14 | 14.5 | 15 | 15.5 | 16 | 16.5 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| neat | 1 | 17337 | 17282 | 17160 | 17053 | 17017 | 18332 | 20694 | 23227 | 26087 | 28711 | 30945 | 32682 | 33918 | 34055 | 33994 |
| neat | 2 | -70 | -192 | -64 | 40 | 180 | 525 | 1157 | 2277 | 4074 | 7422 | 12220 | 18314 | 25041 | 30863 | 34837 |
| neat | 3 | 7016 | 10999 | 16022 | 21256 | 25928 | 31071 | 35029 | 37980 | 39790 | 40812 | 40940 | 41517 | 41813 | 42057 | 42298 |
| neat | 4 | 2758 | 4376 | 6466 | 9030 | 12308 | 15747 | 19620 | 23798 | 28556 | 32611 | 35434 | 37586 | 38556 | 39286 | 39646 |
| neat | 5 | 280 | 610 | 1110 | 1608 | 2432 | 3201 | 4345 | 5856 | 7751 | 10080 | 12680 | 15582 | 18478 | 21332 | 24295 |
| neat | 6 | 1444 | 2665 | 4044 | 6046 | 8625 | 11674 | 14918 | 18903 | 22691 | 26539 | 30134 | 32551 | 35444 | 37114 | 38481 |
| neat | 7 | -27 | 58 | 122 | 327 | 754 | 1798 | 4068 | 7224 | 11936 | 17594 | 22867 | 28016 | 32542 | 35621 | 37943 |
| -1 | 1 | 119 | 388 | 919 | 1850 | 3019 | 4951 | 7645 | 11546 | 16532 | 22526 | 28749 | 33323 | 36226 | 38191 | 38612 |
| -1 | 2 | 1199 | 1660 | 2539 | 3592 | 4779 | 6415 | 8478 | 11139 | 14795 | 19300 | 24268 | 28715 | 32386 | 34696 | 36430 |
| -1 | 3 | -168 | -259 | -226 | -220 | -241 | -238 | -134 | -140 | -226 | -177 | -213 | -195 | -201 | -213 | -210 |
| -1 | 4 | -326 | -314 | -253 | -311 | -152 | -210 | -286 | -290 | -201 | -250 | -213 | -119 | -277 | -271 | -271 |
| -1 | 5 | -244 | -244 | -165 | -244 | -92 | -247 | -250 | -235 | -247 | -232 | -229 | -159 | -177 | -220 | -220 |
| -1 | 6 | -152 | 86 | 76 | 354 | 852 | 1566 | 2704 | 4087 | 5851 | 8026 | 10685 | 13733 | 17566 | 21293 | 24982 |
| -1 | 7 | -286 | -244 | -250 | -219 | -161 | -6 | 336 | 712 | 1725 | 3544 | 6226 | 10880 | 17005 | 22904 | 27803 |
| -2 | 1 | -204 | -241 | -128 | -238 | -232 | -204 | -226 | -241 | -220 | -119 | -229 | -220 | -67 | -210 | -82 |
| -2 | 2 | -241 | -241 | -174 | -95 | -220 | -232 | -140 | -232 | -211 | -220 | -67 | -229 | -226 | -223 | -177 |
| -2 | 3 | -143 | -186 | -165 | -168 | -97 | -180 | -143 | -174 | -177 | -168 | -171 | -177 | -174 | -174 | -162 |
| -2 | 4 | -186 | -177 | -171 | -180 | -113 | -40 | -180 | -144 | -177 | -125 | -43 | -150 | -171 | -153 | -159 |
| -2 | 5 | -317 | -314 | -220 | -317 | -241 | -320 | -299 | -311 | -311 | -308 | -305 | -186 | -207 | -311 | -253 |
| -2 | 6 | -213 | -76 | -216 | -195 | -198 | -210 | -109 | -204 | -204 | -186 | -207 | -64 | -192 | -189 | -195 |
| -2 | 7 | -314 | -287 | -323 | -314 | -339 | -247 | -336 | -244 | -333 | -308 | -323 | -326 | -278 | -317 | -323 |
| -3 | 1 | -214 | -217 | -153 | -214 | -89 | -223 | -174 | -202 | -159 | -202 | -223 | -208 | -202 | -113 | -214 |
| -3 | 2 | -204 | -210 | -143 | -61 | -174 | -158 | -55 | -204 | -155 | -235 | -88 | -204 | -204 | -210 | -73 |
| -3 | 3 | -168 | -171 | -180 | -192 | -31 | -177 | -174 | -180 | -168 | -174 | -55 | -180 | -140 | -61 | -31 |
| -3 | 4 | -180 | -180 | -165 | -180 | -156 | -174 | -171 | -79 | -180 | -95 | -37 | -156 | -153 | -128 | -150 |
| -3 | 5 | -327 | -339 | -223 | -333 | -318 | -327 | -278 | -315 | -306 | -293 | -315 | -190 | -187 | -330 | -171 |
| -3 | 6 | -213 | -94 | -216 | -73 | -198 | -219 | -140 | -213 | -204 | -73 | -210 | -70 | -204 | -201 | -55 |
| -3 | 7 | -186 | -149 | -223 | -189 | -226 | -76 | -229 | -64 | -216 | -213 | -201 | -216 | -204 | -189 | -204 |
| -4 | 1 | -388 | -351 | -376 | -312 | -370 | -370 | -385 | -330 | -391 | -385 | -254 | -245 | -382 | -379 | -385 |
| -4 | 2 | -207 | -207 | -125 | -52 | -137 | -107 | -204 | -165 | -46 | -204 | -195 | -183 | -183 | -131 | -195 |
| -4 | 3 | -162 | -168 | -171 | -180 | -37 | -28 | -174 | -171 | -28 | -171 | -31 | -174 | -9 | -52 | -98 |
| -4 | 4 | -196 | -174 | -183 | -196 | -196 | -183 | -186 | -180 | -180 | -76 | -134 | -168 | -153 | -37 | -46 |
| -4 | 5 | -199 | -235 | -77 | -226 | -232 | -205 | -92 | -134 | -107 | -131 | -199 | -116 | -165 | -183 | -247 |
| -4 | 6 | -213 | -174 | -247 | -79 | -232 | -235 | -219 | -244 | -91 | -79 | -247 | -140 | -238 | -100 | -174 |
| -4 | 7 | -131 | -125 | -220 | -159 | -226 | -183 | -201 | -226 | -210 | -214 | -171 | -204 | -214 | -91 | -107 |
| -5 | 1 | -201 | -116 | -247 | -113 | -256 | -122 | -250 | -247 | -244 | -247 | -147 | -159 | -250 | -235 | -119 |
| -5 | 2 | -217 | -211 | -134 | -174 | -119 | -40 | -205 | -58 | -174 | -202 | -183 | -168 | -186 | -73 | -186 |
| -5 | 3 | -339 | -219 | -326 | -332 | -268 | -235 | -332 | -253 | -277 | -320 | -262 | -308 | -201 | -274 | -290 |
| -5 | 4 | -214 | -205 | -196 | -217 | -205 | -214 | -162 | -208 | -177 | -61 | -196 | -190 | -125 | -147 | -28 |
| -5 | 5 | -177 | -250 | -107 | -229 | -247 | -131 | -220 | -198 | -171 | -119 | -128 | -241 | -232 | -128 | -247 |
| -5 | 6 | -263 | -214 | -297 | -171 | -284 | -187 | -290 | -147 | -193 | -135 | -293 | -199 | -284 | -211 | -269 |
| -5 | 7 | -86 | -101 | -235 | -132 | -211 | -232 | -174 | -229 | -165 | -229 | -77 | -205 | -226 | -135 | -184 |
| neg | 1 | -101 | -162 | -235 | -177 | -229 | -202 | -61 | -220 | -64 | -67 | -202 | -195 | -128 | -55 | -186 |
| neg | 2 | -180 | -192 | -232 | -208 | -204 | -217 | -143 | -107 | -201 | -122 | -217 | -208 | -110 | -183 | -217 |
| neg | 3 | -375 | -378 | -378 | -381 | -225 | -372 | -372 | -320 | -381 | -369 | -384 | -378 | -314 | -375 | -326 |
| neg | 4 | -308 | -305 | -293 | -311 | -186 | -213 | -302 | -293 | -302 | -293 | -293 | -305 | -277 | -308 | -171 |
| neg | 5 | -275 | -278 | -202 | -266 | -202 | -122 | -272 | -263 | -260 | -266 | -260 | -272 | -247 | -266 | -226 |
| neg | 6 | -266 | -260 | -174 | -254 | -211 | -211 | -245 | -251 | -101 | -260 | -135 | -248 | -260 | -147 | -248 |

**Appendix 5.9 (Cont'd..): Zeroed fluorescence data of EHC-GAL and 4-MU-GAL with final sewage sample over 18 h.**

**4-MU-GAL**

| Dilution | Replicate | 17.5 | 18 |
|---|---|---|---|
| neat | 1 | 33997 | 33958 |
| neat | 2 | 36827 | 37687 |
| neat | 3 | 42387 | 42579 |
| neat | 4 | 40494 | 40561 |
| neat | 5 | 27316 | 29798 |
| neat | 6 | 39189 | 39912 |
| neat | 7 | 39445 | 40849 |
| -1 | 1 | 39207 | 39662 |
| -1 | 2 | 37446 | 37998 |
| -1 | 3 | -204 | -104 |
| -1 | 4 | -207 | -265 |
| -1 | 5 | -61 | -202 |
| -1 | 6 | 28617 | 31440 |
| -1 | 7 | 31471 | 34041 |
| -2 | 1 | -217 | -213 |
| -2 | 2 | -86 | -214 |
| -2 | 3 | -15 | -162 |
| -2 | 4 | -177 | -162 |
| -2 | 5 | -311 | -311 |
| -2 | 6 | -192 | -125 |
| -2 | 7 | -302 | -317 |
| -3 | 1 | -83 | -220 |
| -3 | 2 | -207 | -183 |
| -3 | 3 | -140 | -177 |
| -3 | 4 | -174 | -134 |
| -3 | 5 | -336 | -339 |
| -3 | 6 | -216 | -186 |
| -3 | 7 | -100 | -219 |
| -4 | 1 | -379 | -245 |
| -4 | 2 | -201 | -140 |
| -4 | 3 | -162 | -171 |
| -4 | 4 | -119 | -58 |
| -4 | 5 | -192 | -232 |
| -4 | 6 | -262 | -225 |
| -4 | 7 | -214 | -186 |
| -5 | 1 | -247 | -226 |
| -5 | 2 | -177 | -52 |
| -5 | 3 | -308 | -302 |
| -5 | 4 | -25 | -37 |
| -5 | 5 | -104 | -223 |
| -5 | 6 | -132 | -287 |
| -5 | 7 | -223 | -132 |
| neg | 1 | -119 | -208 |
| neg | 2 | -211 | -211 |
| neg | 3 | -381 | -274 |
| neg | 4 | -253 | -171 |
| neg | 5 | -189 | -214 |
| neg | 6 | -241 | -235 |

## Appendix 5.10: Unconfirmed and confirmed MPN values for four sewage samples over 18 h assay with EHC-GAL, 4-MU-GAL and LTB.

**Raw sewage sample**

| | 0 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 | 11.5 | 12.0 | 12.5 | 13.0 | 13.5 | 14.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EHC-GAL unconfirmed | 0 | 0 | 0 | 0 | 10.3 | 23.0 | 23.0 | 23.0 | 23.0 | 23.0 | 23.0 | 52.1 | 354.5 | 354.5 | 832.9 | 832.9 | 1335.8 | 1793.5 | 2564.7 | 2977.6 | 2977.6 | 3508.8 | 4015.4 | 13399.1 |
| EHC-GAL confirmed | 0 | 0 | 0 | 0 | 7.2 | 10.3 | 10.3 | 10.3 | 10.3 | 10.3 | 10.3 | 18.2 | 24.5 | 24.5 | 27.6 | 27.6 | 30.8 | 34.3 | 37.8 | 41.4 | 41.4 | 44.4 | 45.1 | 45.1 |
| 4-MU-GAL unconfirmed | 0 | 0 | 0 | 0 | 0 | 10.3 | 10.3 | 14.8 | 14.8 | 14.8 | 14.8 | 52.1 | 103.0 | 230.3 | 230.3 | 385.2 | 665.1 | 915.3 | 1261.7 | 2568.2 | 4924.5 | 6712.1 | 6712.1 | 6712.1 |
| 4-MU-GAL confirmed | 0 | 0 | 0 | 0 | 0 | 10.3 | 10.3 | 14.8 | 14.8 | 14.8 | 14.8 | 52.1 | 103.0 | 147.6 | 147.6 | 218.7 | 261.5 | 310.8 | 368.3 | 497.8 | 497.8 | 564.1 | 564.1 | 564.1 |

**Settled sewage sample**

| | 0 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 | 11.5 | 12.0 | 12.5 | 13.0 | 13.5 | 14.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EHC-GAL unconfirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.4 | 7.2 | 23.0 | 23.0 | 37.0 | 67.0 | 133.5 | 256.2 | 358.6 | 358.6 | 915.3 | 1261.7 | 1261.7 | 1261.7 | 1519.6 | 1519.6 |
| EHC-GAL confirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.0 | 10.3 | 10.3 | 15.2 | 20.9 | 30.4 | 38.5 | 46.1 | 46.1 | 51.1 | 51.1 | 51.1 | 51.1 | 55.2 | 55.2 |
| 4-MU-GAL unconfirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.0 | 14.8 | 14.8 | 29.5 | 38.5 | 147.6 | 181.0 | 218.7 | 520.9 | 520.9 | 520.9 | 520.9 | 520.9 | 520.9 | 520.9 |
| 4-MU-GAL confirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.4 | 5.0 | 5.0 | 9.2 | 11.3 | 20.7 | 23.2 | 25.6 | 31.2 | 31.2 | 31.2 | 31.2 | 31.2 | 31.2 | 31.2 |

**Filtered sewage sample**

| | 0 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 | 11.5 | 12.0 | 12.5 | 13.0 | 13.5 | 14.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EHC-GAL unconfirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.4 | 4.9 | 10.3 | 18.1 | 29.5 | 49.0 | 49.0 | 49.0 | 49.0 | 92.3 | 112.1 | 112.1 | 112.1 | 112.1 |
| EHC-GAL confirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.4 | 3.0 | 7.2 | 7.2 | 10.3 | 15.2 | 15.2 | 15.2 | 15.2 | 17.9 | 20.6 | 20.6 | 20.6 | 20.6 |
| 4-MU-GAL unconfirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.0 | 9.2 | 21.5 | 21.5 | 21.5 | 83.3 | 83.3 | 151.8 | 215.1 | 369.5 | 369.5 | 490.2 | 490.2 |
| 4-MU-GAL confirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.0 | 9.2 | 21.5 | 21.5 | 21.5 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 40.1 | 40.1 |

**Final sewage sample**

| | 0 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 | 11.5 | 12.0 | 12.5 | 13.0 | 13.5 | 14.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EHC-GAL unconfirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.0 | 3.0 | 7.2 | 7.2 | 12.7 | 12.7 | 29.5 | 37.0 | 37.0 | 67.0 | 67.0 |
| EHC-GAL confirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.0 | 3.0 | 7.2 | 7.2 | 12.7 | 12.7 | 18.1 | 18.1 | 18.1 | 26.2 | 26.2 |
| 4-MU-GAL unconfirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.0 | 3.0 | 6.6 | 6.6 | 8.4 | 8.4 | 11.3 | 26.2 | 52.1 | 52.1 | 72.9 |
| 4-MU-GAL confirmed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.0 | 3.0 | 6.6 | 6.6 | 8.4 | 8.4 | 11.3 | 26.2 | 52.1 | 52.1 | 72.9 |

## Appendix 5.10 (Cont'd..): Unconfirmed and confirmed MPN values for four sewage samples over 18 h assay with EHC-GAL, 4-MU-GAL and LTB.

| **Raw sewage sample** | | | | | | | | | **Raw sewage sample** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **14.5** | **15.0** | **15.5** | **16.0** | **16.5** | **17.0** | **17.5** | **18.0** | | **18 h LTB MPN** |
| EHC-GAL unconfirmed | 13399.1 | 13399.1 | 13399.1 | 13399.1 | 13399.1 | 13399.1 | 15699.4 | 15699.4 | LTB unconfirmed | 10194.2 |
| EHC-GAL confirmed | 49.0 | 49.0 | 49.0 | 49.0 | 49.0 | 49.0 | 49.0 | 49.0 | LTB confirmed | 33.9 |
| 4-MU-GAL unconfirmed | 6712.1 | 6712.1 | 6712.1 | 6712.1 | 6712.1 | 6712.1 | 6712.1 | 6712.1 | | |
| 4-MU-GAL confirmed | 564.1 | 564.1 | 564.1 | 564.1 | 564.1 | 564.1 | 564.1 | 564.1 | | |
| **Settled sewage sample** | | | | | | | | | **Settled sewage sample** | |
| | **14.5** | **15.0** | **15.5** | **16.0** | **16.5** | **17.0** | **17.5** | **18.0** | | |
| EHC-GAL unconfirmed | 2153.5 | 2153.5 | 2153.5 | 2568.2 | 2568.2 | 3044.3 | 3044.3 | 3044.3 | LTB unconfirmed | 2378.8 |
| EHC-GAL confirmed | 55.2 | 55.2 | 55.2 | 59.4 | 59.4 | 59.4 | 59.4 | 59.4 | LTB confirmed | 468.0 |
| 4-MU-GAL unconfirmed | 520.9 | 1030.5 | 1259.9 | 1259.9 | 1259.9 | 1782.2 | 1782.2 | 1782.2 | | |
| 4-MU-GAL confirmed | 31.2 | 36.6 | 36.6 | 36.6 | 36.6 | 39.5 | 39.5 | 39.5 | | |
| **Filtered sewage sample** | | | | | | | | | **Filtered sewage sample** | |
| | **14.5** | **15.0** | **15.5** | **16.0** | **16.5** | **17.0** | **17.5** | **18.0** | | |
| EHC-GAL unconfirmed | 151.8 | 151.8 | 177.0 | 177.0 | 251.6 | 251.6 | 251.6 | 251.6 | LTB unconfirmed | 181.0 |
| EHC-GAL confirmed | 20.6 | 20.6 | 20.6 | 20.6 | 23.8 | 23.8 | 23.8 | 23.8 | LTB confirmed | 92.3 |
| 4-MU-GAL unconfirmed | 490.2 | 490.2 | 617.5 | 617.5 | 832.9 | 1335.8 | 1335.8 | 1335.8 | | |
| 4-MU-GAL confirmed | 40.1 | 40.1 | 45.4 | 45.4 | 51.2 | 51.2 | 51.2 | 51.2 | | |
| **Final sewage sample** | | | | | | | | | **Final sewage sample** | |
| | **14.5** | **15.0** | **15.5** | **16.0** | **16.5** | **17.0** | **17.5** | **18.0** | | |
| EHC-GAL unconfirmed | 67.0 | 92.3 | 92.3 | 92.3 | 92.3 | 92.3 | 92.3 | 92.3 | LTB unconfirmed | 66.5 |
| EHC-GAL confirmed | 26.2 | 26.2 | 26.2 | 26.2 | 26.2 | 26.2 | 26.2 | 26.2 | LTB confirmed | 30.4 |
| 4-MU-GAL unconfirmed | 72.9 | 72.9 | 72.9 | 72.9 | 72.9 | 72.9 | 72.9 | 72.9 | | |
| 4-MU-GAL confirmed | 72.9 | 72.9 | 72.9 | 72.9 | 72.9 | 72.9 | 72.9 | 72.9 | | |